Supplementary information for the manuscript

"**Asymmetric β-methylation of L- and D-*α*-amino acids by a self-contained enzyme cascade**"

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Supplementary Methods

General information

All enzyme-encoding pET28a-based expression plasmids were purchased from BioCat GmbH. The expression strain *E. coli* Δmtn (DE3) was constructed in previous study in our lab.[1] Proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions. The gels were stained with Coomassie brilliant blue.

High resolution electron spray ionization mass spectroscopy (HRESIMS) spectra were obtained on a Bruker maXis 4G UHR-TOF Mass Spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance Neo NMR spectrometer operating at 500 MHz proton frequency and chemical shifts were internally referenced to residual proton signals of solvents. Chemical shifts (δ) were reported in parts per million (ppm). Standard abbreviations indicating multiplicity were used as follows: s (singlet), d (doublet), t (triplet), and m (multiplet). Coupling constants (J) were reported in Hertz (Hz).

Unless otherwise noted, all chemicals and reagents were purchased from Sigma Aldrich and used without further purification. Antibiotics were purchased from PanReac AppliChem. Ingredients for buffers were purchased from Acros Organics. Substrates and authentic compounds were purchased from Fluchem, Alfa Aesar or Acros Organics. L-2-amino-5-methyl hexenoic acid was from Activate Scientific. Deuterated solvents were purchased from Cambridge Isotope Laboratories.

Protein expression

E. coli Δmtn (DE3) cells were transformed with pET28a expression plasmids by electroporation. Transformed cells were first cultivated on solid medium (LB-AGAR plates with 50 µg/ml kanamycin) and then in liquid medium. After incubation at 37°C overnight, 15 ml of pre-culture was used to inoculate fresh Terrific Broth (TB) medium (1L) with kanamycin (50 µg/ml). The cells were grown in 3L shaking flask at 37 °C (170 rpm) until OD600 reached 1.0. The culture was cooled to 20 °C supplemented with 0.1 mM isopropyl β-D-thiogalactoside (IPTG). This culture was incubated at 20 °C for 24 hours. Cells were harvested by centrifugation at 10,000 x g for 30 minutes and stored at -20 °C.

For purification, cell pellets (10 g) were suspended in 40 ml lysis buffer (50 mM sodium phosphate, 300 mM NaCl, 10 mM imidazole, pH 8.0). Cells were disrupted by sonication for 3 x 60 s with Branson sonifier 450 (output control 5, 50% duty cycle). Lysates were centrifuged at 10,000 x g for 1.0 h at 4 °C. The cleared lysate was mixed with 1 ml of Ni^{II} NTA agarose at 4 °C for 20 min and loaded onto a column. The agarose beads were washed with 10 ml lysis buffer containing 10 mM and 20 mM imidazole respectively. The protein was eluted in a lysis buffer solution containing 250 mM imidazole. Protein containing fractions were collected and dialyzed against dialysis buffer (100 mM sodium phosphate, pH 8), aliquoted and stored at -80 °C.

Protein sequences

HMT (GenBank: ABE28953.1)

MGHHHHHHAENLYFQGSGSDPTQPAVPDFETRDPNSPAFWDERFERRFTPWDQAGVPAAFQSFAARHS
GAAVLIPGCGSAYEAVWLAGQGNPVRAIDFSPAAVAAAHEQLGAQHAQLVEQADFFTYEPPFTPAWIY
ERAFLCALPLARRADYAHRMADLLPGGALLAGFFFLGATPKGPPFGIERAELDALLTPYFDLIEDEAV
HDSIAVFAGRERWLTWRRRA

MarG (GenBank: AHJ60976.1)

MGPAAGKTFNTSIAGADDLIRLHLSESPHGASKAALQAAERELARVNVYPDPERQELVRALAAHWGVG
PEHIAVANGSDELVLATALTLGDRNLPGLVTDGTFPGYRACLELLGRGCTAVPPDGTAVDVAGFAARL
PGHGIGYLCNPHNPSGAALTRQELAALVEVSGRSGVPLVFDEAYMEFAGPDVPQTRDLTAAGDAPVVA
LRTFSKAYGLAALRVGYAVGRPDLIAGLRGTLRALPFSVNRLAQAAAIAALGDPDFVDGVRRSTAERR
RWFVGELDRRGRAHLPSVTNFVAVAARDCARAQDRLAADFGILVRNAGLFGFPGYLRTSLGEKKDLER
FLDALDEIEQNPLEHHHHHH

MarI (GenBank: AHJ60978.1)

MGHHHHHHAENLYFQGSGTAPLSRDGLRAMGESVFRPAEWQGAAHTPLDADTAFNGFISTHVVFALEQ
LGLFAWFDESDRLDVPQYCWRRKLDERVFRQLVSAAEAFGYLDVHDDLVTPTPAWSELRRKIGFFTWG
VGGYHDVFANAASIARGERAFGKDVLRDEAMVALGSAQADMALMRDLLDEQIAALDFSVIADLGSGIS
ERVCRLVKSRPGARGLGVDISASATALAAGTVERHELADRVQPICADVLDVLFHGRRIEGADQVDVAM
SFMFLHDLLVDPTTRTDVIPALRKAFPRAHTFLLADTTVRPRDEKDTLPVFSSGFELAHALMGVPIYT
REEYENLFHEGGLHLRRTVPFGAPHTYLFVLEAQ

IlvE (GenBank: MJH26684.1)

MGTKKADYIWFNGEMVRWEDAKVHVMSHALHYGTSVFEGIRCYDSHKGPVVFRHREHMQRLHDSAKIY
RFPVSQSIDELMEACRDVIRKNNLTSAYIRPLIFVGDVGMGVNPPAGYSTDVIIAAFPWGAYLGAEAL
EQGIDAMVSSWNRAAPNTIPTAAKAGGNYLSSLLVGSEARRHGYQEGIALDVNGYISEGAGENLFEVK
DGVLFTPPFTSSALPGITRDAIIKLAKELGIEVREQVLSRESLYLADEVFMSGTAAEITPVRSVDGIQ
VGEGRCGPVTKRIQQAFFGLFTGETEDKWGWLDQVNQLEHHHHHH

SgvM (GenBank: AGN74875.1)

MGHHHHHHAENLYFQGSGMATHDIAAQHLADGIAASGPAPDLAAAAAFLEMGDRLGVVAHLDPDRTLE
TAEVAAALDLPEPALVRYLDAVESAGLVIREGEGRYRACPDFDTIRHQAGYISWTMNANRPFIENARD
FFTDWDKAARTHVRDYREVAVSSQWMGSHAFYPTALATIIDAAPRKVVDLGAGTCRLLIEVLGAVPGS
TGVGLDFAADACRAAEQAVAQAGMTDRLTVVERTIQSVATDPGVLEGADVIHAGFVFHDMLPEEEDVC
DQVLANCRESLAPGGFLAITDAVPYLRNDRERRFSAAVSYYHGEFMRRRLQSEEEWVERLRGAGFSDV
RALTLAFPTGRLFLAHR

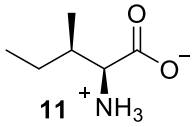
Determination of Conversion efficiency and diastereomeric ratio.

L-amino acids: 1 ml reactions containing 2 mM of substrate, 4 mM methyl iodide, 40 µM SAH, 40 µM PLP, 40 µM IlvE, 40 µM α-keto acid *C*-methyltranferase, 20 µM HMT and 100 mM sodium phosphate buffer (pH = 8.0) were incubated at room temperature. After 24 hours, the reaction was quenched by addition of 500 µl of $CHCl_3$ and vigorous mixing on the vortex. The aqueous layer was isolated by centrifugation and lyophilized. After lyophilization, the mixture was dissolved in 600 µl of D_2O. ^1H NMR was recorded. Conversion efficiency (% of consumed amino acid) and ratio of different product were determined by integration of characteristic ^1H NMR resonances from different compounds (Supplementary Figure 3-23)

D-amino acids: 1 ml reactions containing 2 mM of substrate, 4 mM methyl iodide, 40 µM SAH, 80 µM PLP, 80 µM D-TA, 40 µM α-keto acid *C*-methyltranferase, 20 µM HMT and 100 mM sodium phosphate buffer (pH = 8.0) were incubated at room temperature. Work-up and analysis of these reactions was identical as described above for L-amino acids (Supplementary Figure 24-41).

Characterization of Products

For the structural characterization of products 3 ml reactions were incubated for 24 – 72 h, lyophilized, dissolved in D_2O and analyzed by ^1H NMR, ^{13}C NMR and HRESIMS. Comparison of enzymatically produced L-allo-isoleucine (**11**) and D-isoleucine (**28**) with authentic material established their absolute stereochemistry.

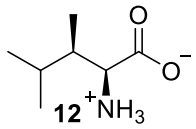


L-*allo*-isoleucine (**2**) from L-norvaline (**1**)

HRESIMS $C_6H_{14}NO_2$ $(M+H)^+$ calcd. 132.1019, obsvd. 132.1018.

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.73 (d, *J* = 3.6 Hz, 1H), 2.03-2.08 (m, 1H), 1.39-1.46 (m, 1H), 1.30-1.36 (m, 1H), 0.95 (t, *J* = 7.3 Hz, 3H), 0.93 (d, *J* = 7.0 Hz, 3H).

Authentic L-*allo*-isoleucine, ^{1}H NMR (500 MHz, D_2O, pD 8) δ 3.74 (d, *J* = 3.6 Hz, 1H), 2.05 – 2.10 (m, 1H), 1.43 (dq, *J* = 14.2, 7.2 Hz, 1H), 1.35 (dq, *J* = 14.2, 7.2 Hz, 1H), 0.97 (t, *J* = 7.4 Hz, 3H), 0.94 (d, *J* = 7.2 Hz, 3H).

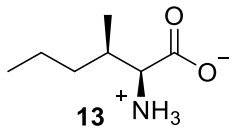


12 from L-leucine (**2**)

HRESIMS $C_7H_{16}NO_2$ $(M+H)^+$ calcd. 146.1176, obsvd. 146.1177.

^{1}H NMR (500 MHz, D_2O, pD 8): δ = 3.80 (d, *J* = 3.8 Hz, 1H), 1.78~1.85 (m, 1H), 1.51~1.58 (m, 1H), 0.99 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.5 Hz, 3H), 0.90 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 176.6 (assigned by 2D NMR), 57.5, 40.9, 29.3, 20.3, 19.4, 10.9.

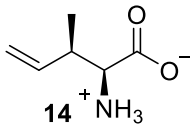


13 from L-norleucine (**3**)

HRESIMS $C_7H_{16}NO_2$ $(M+H)^+$ calcd. 146.1176, obsvd. 146.1177.

^{1}H NMR (500 MHz, D_2O, pD 8): δ = 3.72 (d, *J* = 3.6 Hz, 1H), 2.17-2.22 (m. 1H), 1.28-1.43 (m, 4H), 0.92 (d, *J* = 7.0 Hz, 3H), 0.91 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.6 (assigned by 2D NMR), 58.81, 34.54, 33.30, 19.60, 13.51, 13.10.

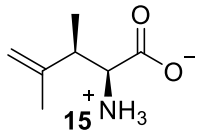


14 from L-allylglycine (**4**)

HRESIMS $C_6H_{12}NO_2$ $(M+H)^+$ calcd. 130.0863, obsvd. 130.0861.

^{1}H NMR (500 MHz, D_2O, pD 8): δ = 5.86 (ddd, *J* = 6.6, 10.6, 17.0 Hz, 1H), 5.28 (d *J* = 10.6 Hz 1H), 5.27 (d, *J* = 17.0 Hz, 1H), 3.78 (d, *J* = 4.0 Hz,1H), 2.87-2.93 (m, 1H), 1.11 (d, *J* = 7.1 Hz, 1H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 173.4 (assigned by 2D NMR), 137.6, 117.5, 58.4, 37.8, 13.0.

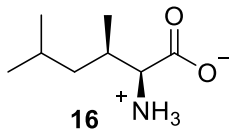


15 from L-4,5-dehydroleucine (**5**). SgvM concentration was 80 μM in this cascade.

HRESIMS $C_7H_{12}NO_2$ $(M-H)^-$, calcd. 142.0874, obsvd. 142.0876.

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 5.07 (s, 1H), 4.93 (s, 1H), 3.88 (d, *J* = 4.1 Hz, 1H), 2.81-2.86 (m, 1H), 1.81 (s, 3H), 1.07 (d, *J* = 7.2 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.1 (assigned by 2D NMR), 144.0 (assigned by 2D NMR), 113.4 (assigned by 2D NMR), 56.2 (assigned by 2D NMR), 40.4, 20.6, 11.8.

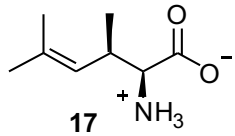


16 from L-2-amino-5-methyl hexanoic acid (**6**)

HRESIMS $C_8H_{16}NO_2$ $(M-H)^-$, calcd. 158.1187, obsvd. 158.1186.

^{1}H NMR (500 MHz, D_2O, pD 8): δ = 3.65 (d, *J* = 3.6 Hz, 1H), 2.22-2.27 (m. 1H), 1.59-1.67 (m. 1H), 1.20 (t, *J* = 7.4 Hz, 2H), 0.90 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.6 Hz, 3H),

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.7 (assigned by 2D NMR), 59.1, 41.5, 31.3, 24.4, 22.2, 21.1, 13.6.

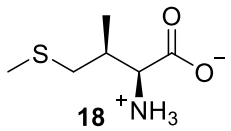


17 from L-2-amino-5-methyl Hex-4-enoic acid (**7**)

HRESIMS $C_8H_{14}NO_2$ $(M-H)^-$, calcd. 156.1030, obsvd. 156.1029.

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 4.96 (d, *J* = 9.7 Hz, 1H), 3.59 (d, *J* = 4.8 Hz, 1H), 2.96-3.01 (m, 1H), 1.68 (d, *J* = 1.4 Hz, 3H), 1.64 (d, *J* = 1.4 Hz, 3H), 1.01 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 173.6 (assigned by 2D NMR), 136.8, 123.0, 59.23, 33.2, 25.0, 17.3, 15.8.



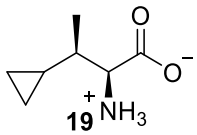
18

18 from L-methionine (**8**). SgvM, PLP and IlvE concentration was 80 μM in this cascade.

HRESIMS $C_6H_{14}NO_2S$ $(M+H)^+$, calcd. 164.0740, obsvd. 164.0739.

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.91 (d, *J* = 3.3 Hz, 1H), 2.58 (d, *J* = , 3.0 Hz, 1H), 2.57 (d, *J* = , 3.1 Hz, 1H), 2.38-2.43 (m, 1H), 2.11 (s, 3H), 1.01 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.3 (assigned by 2D NMR), 57.4, 36.5, 33.2, 14.3, 13.6.



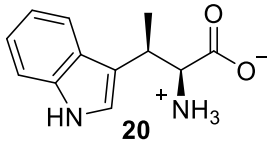
19

19 from L-cyclopropyl alanine (**9**).

HRESIMS $C_7H_{12}NO_2$ $(M-H)^-$, calcd. 142.0874, obsvd. 142.0876.

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.78 (d, *J* = 4.0 Hz, 1H), 1.33–1.44 (m, 1H), 1.03 (d, *J* = 7.1 Hz, 3H), 0.64-0.72 (m, 1H), 0.56-0.62 (m, 1H), 0.50-0.55 (m, 1H), 0.20-0.28 (m, 2H).

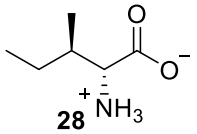
^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.4 (assigned by 2D NMR), 59.6, 39.3, 13.7, 13.6, 3.8, 3.5.



20

20 from L-tryptophan (**10**). MarG and MarI were used for this cascade. ^{1}H NMR is consistent with that from literature.[2]

^{1}H NMR (500 MHz, D_2O, pD 8): δ = 7.79 (d, *J* = 7.9 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 7.34 (brs, 1H), 7.29 (d, *J* = 7.2, 8.2 Hz, 1H), 7.21 (d, *J* = 7.2, 7.9 Hz, 1H), 4.16 (d, *J* = 4.0 Hz, 1H), 3.95 (m, 1H), 1.41 (d, *J* = 7.3 Hz, 3H).

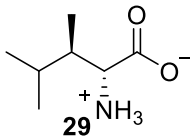


28

28 from D-norvaline (**21**).

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.64 (d, *J* = 4.0 Hz, 1H), 1.93 - 1.98 (m, 1H), 1.42 - 1.47 (m, 1H), 1.21 - 1.28 (m, 1H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H).

Authentic D-isoleucine. ^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.66 (d, *J* = 4.0 Hz, 1H), 1.95 – 2.01 (m, 1H), 1.41 - 1.53 (m, 1H), 1.19 – 1.31 (m, 1H), 1.01 (d, *J* = 7.0 Hz, 3H), 0.94 (t, *J* = 7.4 Hz, 3H).



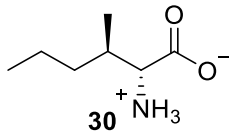
29

29 from D-leucine (**22**).

HRESIMS $C_7H_{15}NNaO_2$ $(M+Na)^+$, calcd. 168.0995, obsvd. 168.0995.

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.68 (d, *J* = 5.4 Hz, 1H), 1.73 - 1.79 (m, 1H), 1.63 - 1.73 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.5 (assigned by 2D NMR), 58.0, 40.8, 28.3, 20.7, 17.7, 16.8, 11.2.



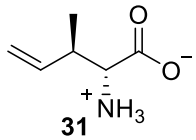
30

30 from D-norleucine (**23**).

HRESIMS $C_7H_{16}NO_2$ $(M+H)^+$, calcd. 146.1176, obsvd. 146.1173.

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.64 (d, *J* = 3.8 Hz, 1H), 2.06 - 2.14 (m, 1H), 1.17 – 1.51 (m, 4H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.86 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.3 (assigned by 2D NMR), 59.4 (assigned by 2D NMR), 33.47, 33.35, 19.63, 15.04, 13.14.



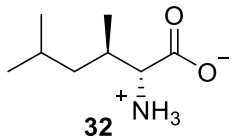
31

31 from D-allylglycine (**24**).

HRESIMS $C_6H_{12}NO_2$ $(M+H)^+$, calcd. 130.0863, obsvd. 130.0863.

^{1}H NMR (500 MHz, D_2O, pD 8.0) δ = 5.75 (ddd, *J* = 17.5, 10.4, 7.3 Hz, 1H), 5.24 (d, *J* = 17.4 Hz, 1H), 5.23 (d, *J* = 10.4 Hz, 1H), 3.61 (d, *J* = 5.6 Hz, 1H), 2.76-2.83 (m, 1H), 1.14 (d, *J* = 7.1 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 173.8 (assigned by 2D NMR), 136.75, 118.13, 59.13, 38.40, 15.30.



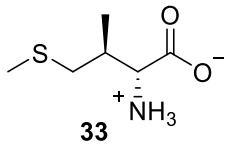
32

32 from D-2-amino-5-methyl hexanoic acid (**25**).

HRESIMS $C_8H_{18}NO_2$ $(M+H)^+$, calcd. 160.1332, obsvd. 160.1330.

^{1}H NMR (500 MHz, D_2O, pD 8): δ = 3.65 (d, *J* = 3.6 Hz, 1H), 2.16-2.21 (m, 1H), 1.61 - 1.69 (m, 1H), 1.17 - 1.23 (m, 1H), 1.10-1.16 (m, 1H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.83 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.1 (assigned by 2D NMR), 60.03, 40.23, 31.63, 24.45, 22.87, 20.57, 15.26.



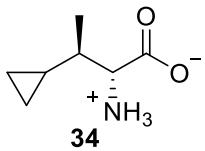
33

33 from D-methionine (**26**).

HRESIMS $C_6H_{14}NO_2S$ $(M+H)^+$, calcd. 164.0740, obsvd. 164.0736.

^{1}H NMR (500 MHz, D_2O, pD 8.0) δ = 3.85 (d, *J* = 3.6 Hz, 1H), 2.71 (dd, *J* = 13.4, 6.4 Hz, 1H), 2.53 (dd, *J* = 13.4, 8.0 Hz, 1H), 2.24 - 2.29 (m, 1H), 2.10 (s, 3H), 1.07 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 173.3 (assigned by 2D NMR), 58.1 (assigned by 2D NMR), 36.66, 33.8, 14.6, 14.4.



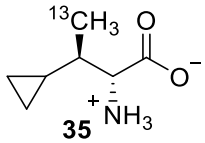
34

34 from D-cyclopropyalanine (**27**).

HRESIMS $C_7H_{13}NNaO_2$ $(M+Na)^+$, calcd. 166.0838, obsvd. 166.0838.

^{1}H NMR (500 MHz, D_2O, pD 8.0) δ = 3.67 (d, *J* = 4.7 Hz, 1H), 1.29 - 1.35 (m, 1H), 1.09 (d, *J* = 7.1 Hz, 3H), 0.65 - 0.71 (m, 1H), 0.46 - 0.53 (m, 2H), 0.22 - 0.26 (m, 1H), 0.10 - 0.14 (m, 1H).

^{13}C NMR (126 MHz, D2O, pD 8) δ = 174.4 (assigned by 2D NMR), 60.0, 39.5, 16.0, 12.8, 3.9, 2.6.



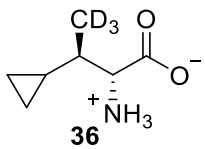
35

35 from D-cyclopropyalanine (**27**) and $^{13}CH_3I$.

HRESIMS $C_6H_{14}{}^{13}CNO_2$ $(M+H)^+$, calcd. 145.1053, obsvd. 145.1055

^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.64 (dd, *J* = 4.5, 3.6 Hz, 1H), 1.35 – 1.26 (m, 1H), 1.05 (dd, *J* = 126.6, 7.0 Hz, 3H), 0.58 – 0.72 (m, 1H), 0.39 – 0.55 (m, 2H), 0.17 – 0.31 (m, 1H), 0.02 – 0.13 (m, 1H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.5 (assigned by 2D NMR), 60.0, 39.5, 16.0, 12.8, 3.9, 2.6.



36

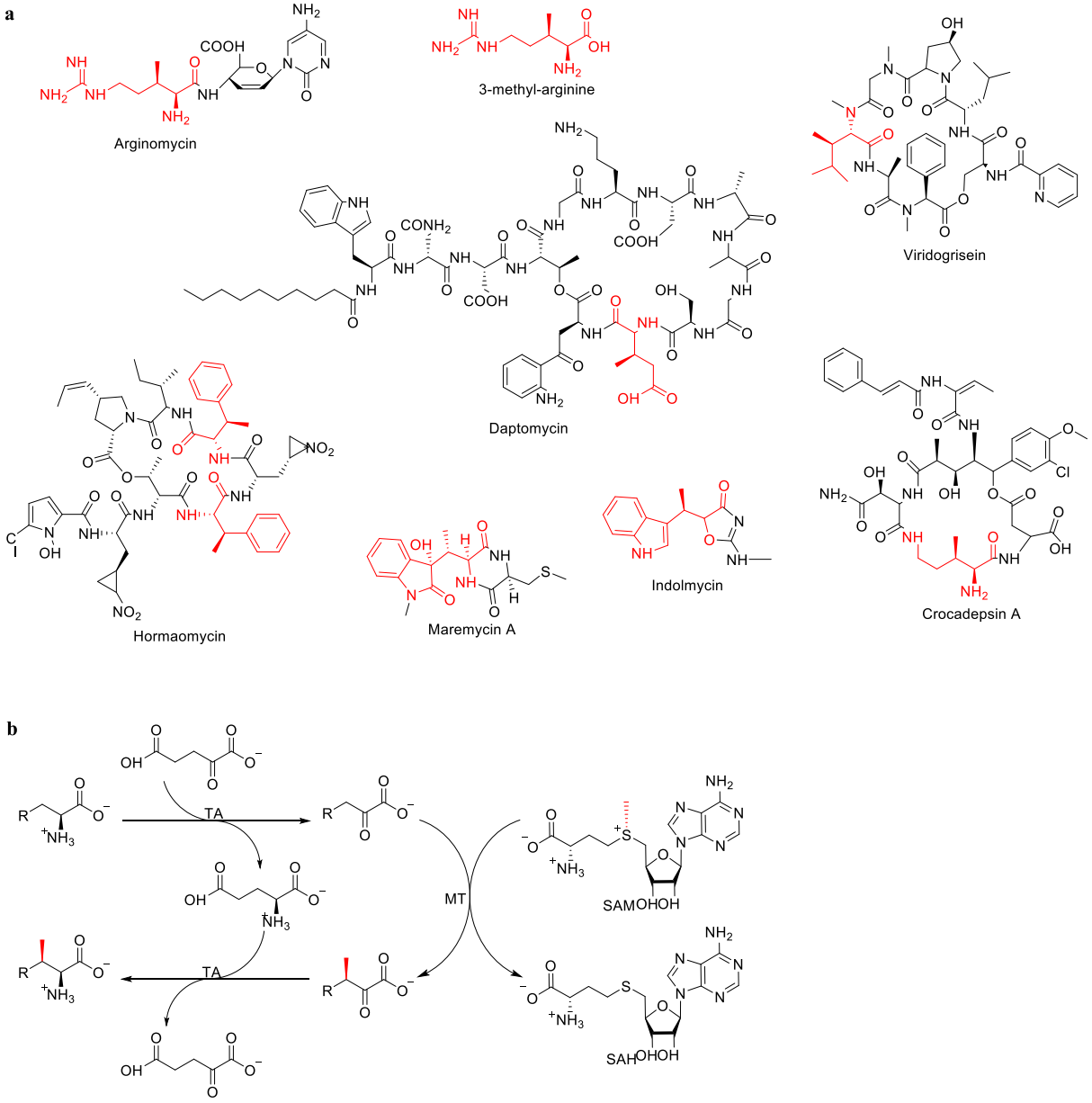
36 from D-cyclopropyalanine (**27**) and CD_3I.

HRESIMS $C_7H_{11}D_3NO_2$ $(M+H)^+$, calcd. 147.1208, obsvd. 147.1207

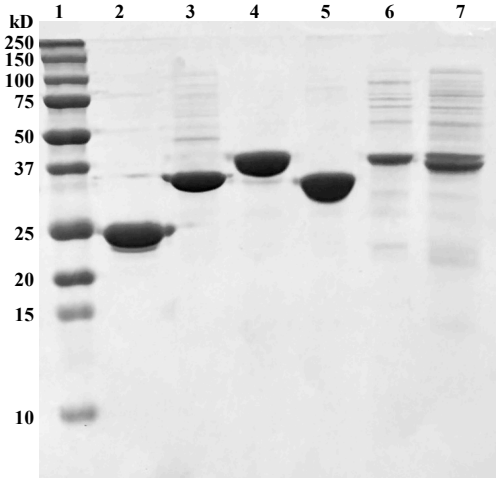
^{1}H NMR (500 MHz, D_2O, pD 8) δ = 3.64 (d, *J* = 4.6 Hz, 1H), 1.28 (dd, *J* = 9.9, 4.7 Hz, 1H), 0.59 – 0.70 (m, 1H), 0.41 – 0.53 (m, 2H), 0.17 – 0.25 (m, 1H), 0.09 (ddd, *J* = 9.2, 4.7, 1.5 Hz, 1H).

^{13}C NMR (126 MHz, D_2O, pD 8) δ = 174.4 (assigned by 2D NMR), 59.9, 39.2, 15.2 (CD_3, assigned by 2D NMR), 12.7, 3.8, 2.6.

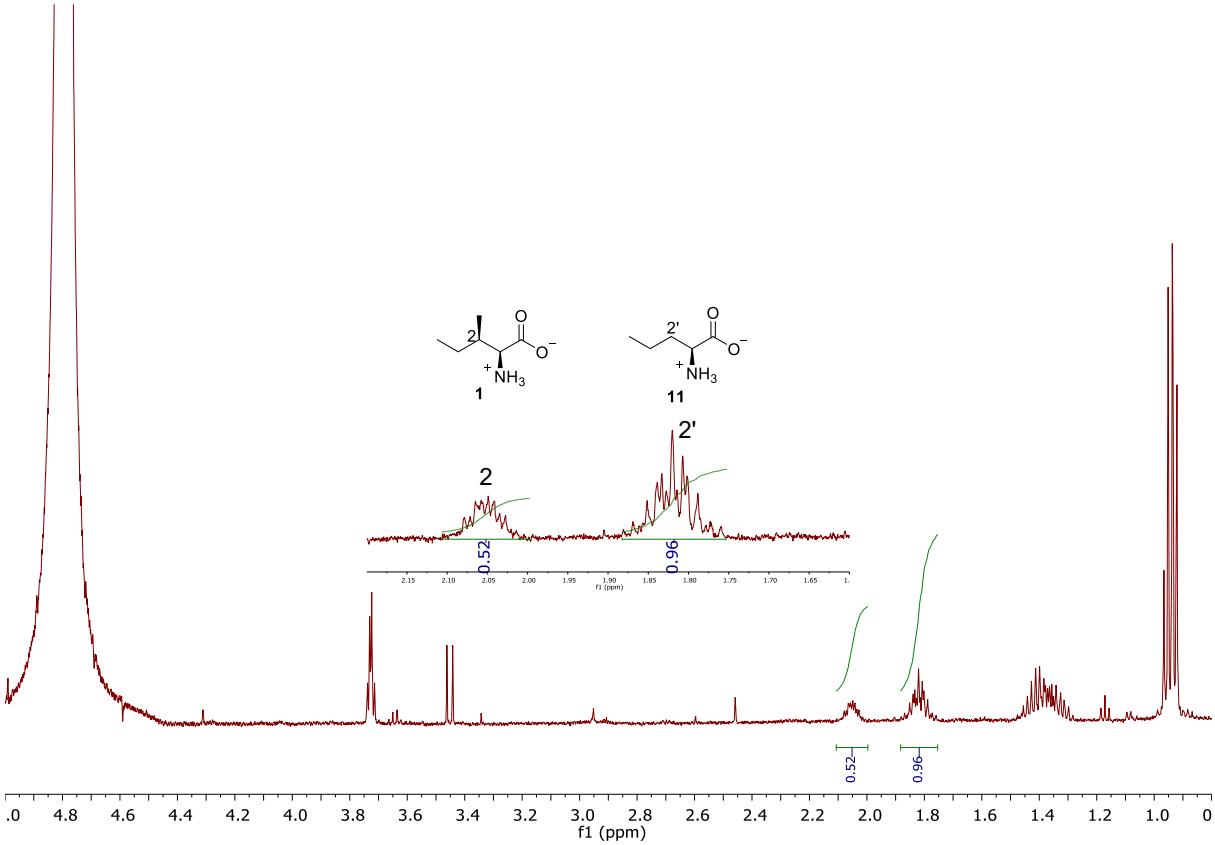
Supplementary Figures



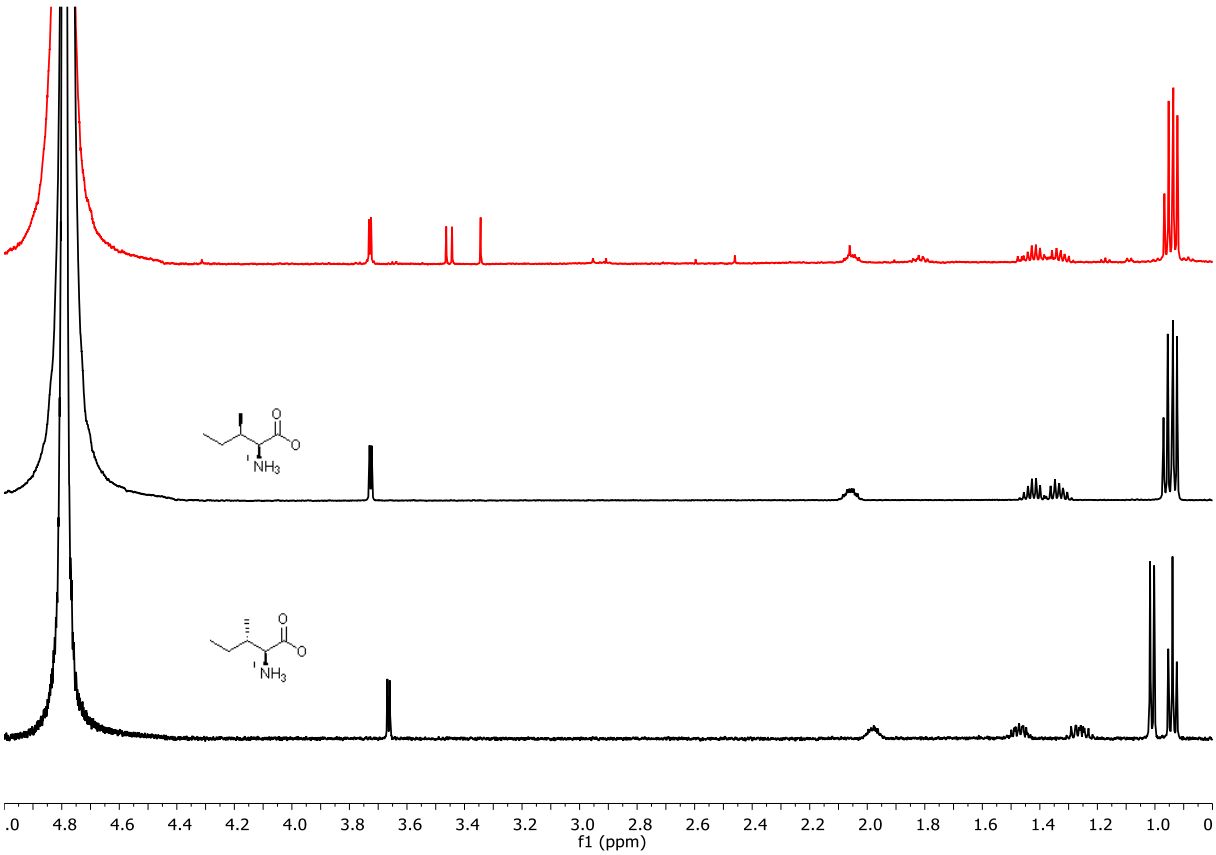
Supplementary Figure 1. Representative natural products containing β-Me-*α*-aa and the biosynthetic pathway of those β-Me-*α*-aa. The existing examples includes β-Me Arg, β-Me Glu, β-Me Trp, β-Me Phe, β-Me Leu.



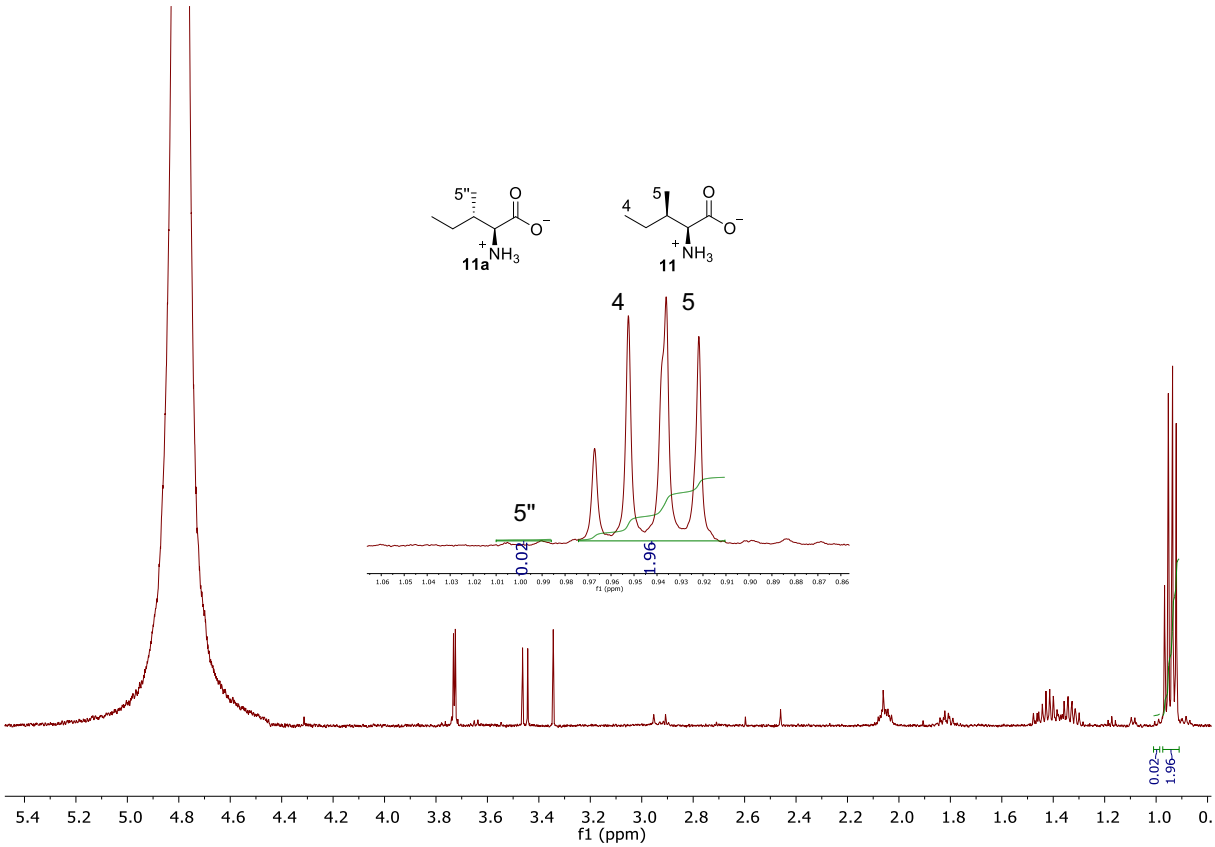
Supplementary Figure 2. SDS-PAGE of recombinant enzymes. lane 1. Marker with annotated MS (kD). lane 2. HMT, lane 3. IlvE, lane 4. SgvM, lane 5. D-TA, lane 6. MarG, lane 7, MarI.



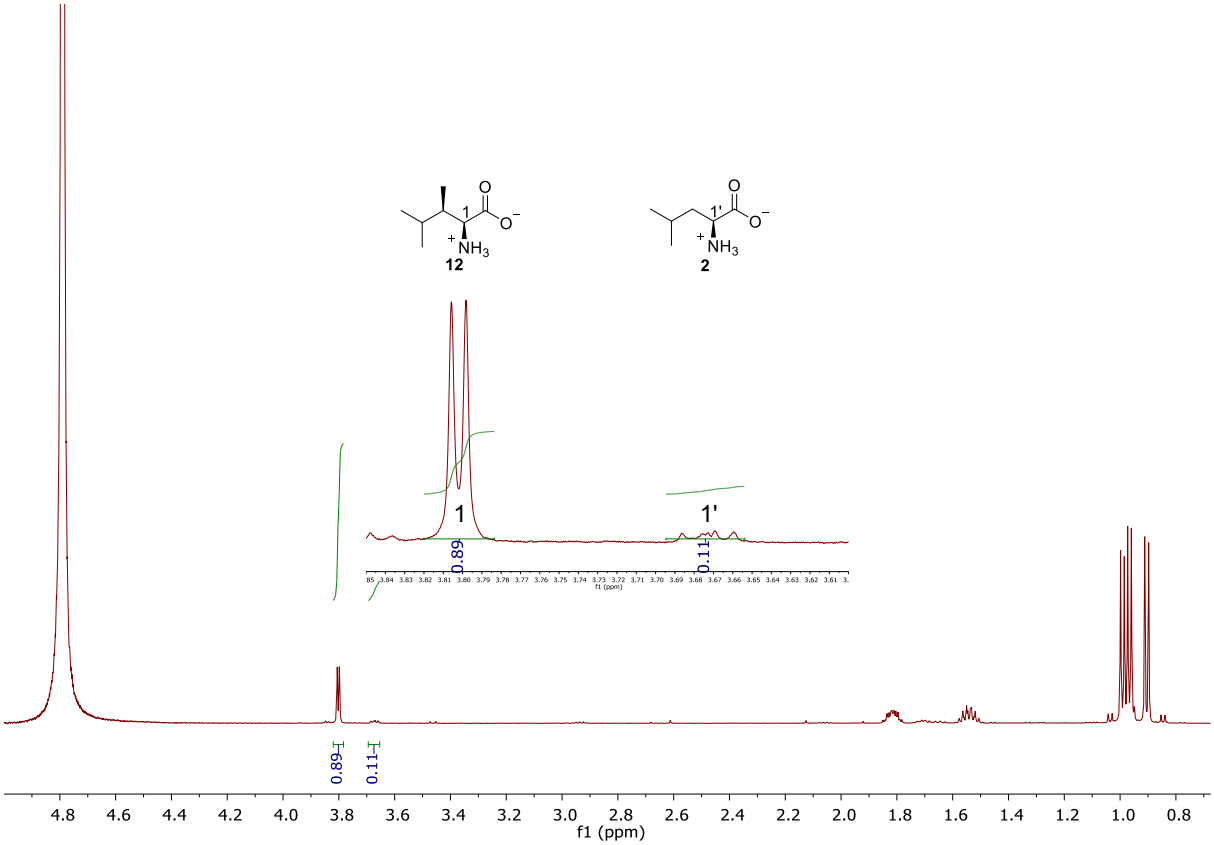
Supplementary Figure 3. Conversion of IlvE-SgvM cascade taking L-norvaline (**1**) as substrate. β-H was choosen to calculate the conversion. Conversion = 0.52/(0.52 + 0.96/2) x 100% = 52%.



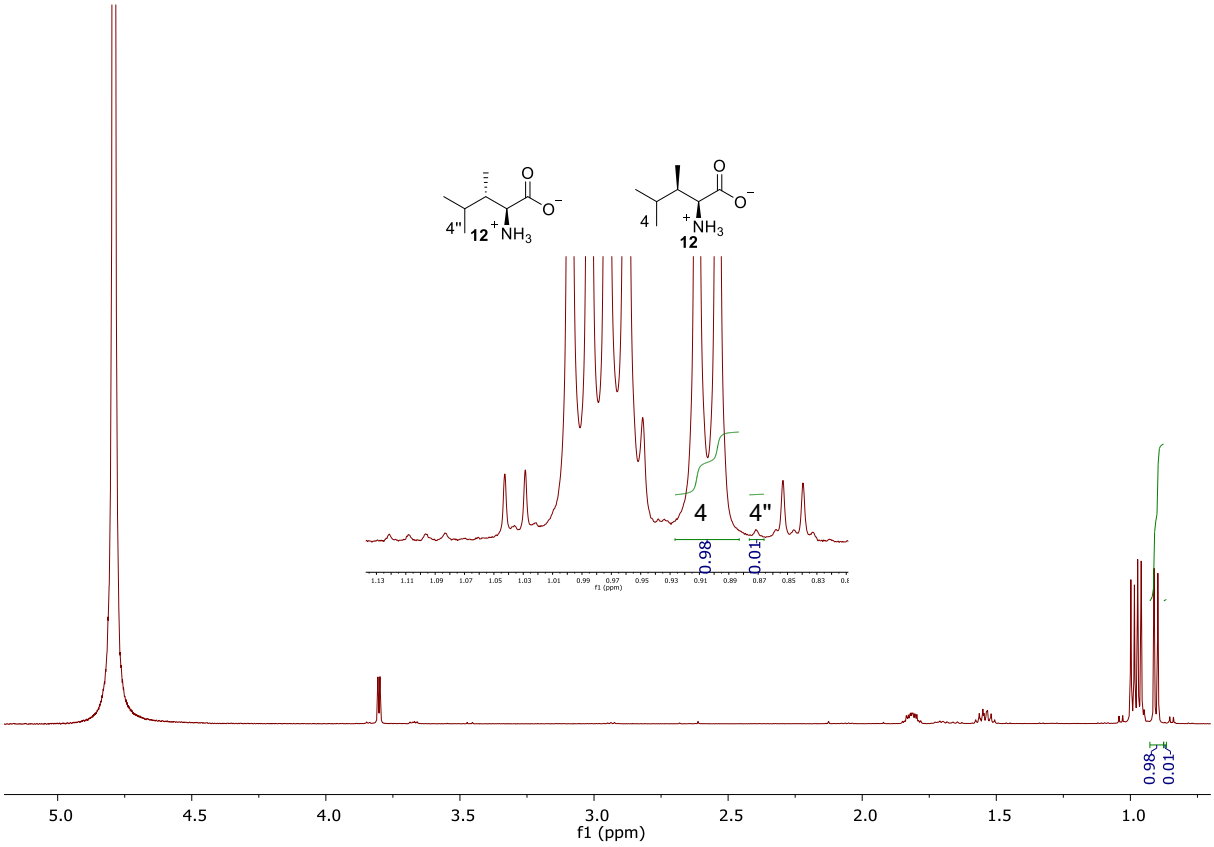
Supplementary Figure 4. ^{1}H NMR of compound **11** (**top**), authentic L-*allo*-isoleucine (**middle**) and L-isoleucine (**bottom**). ^{1}H NMR indicates that enzymatic product has same configurations of 3R as L-*allo*-isoleucine.



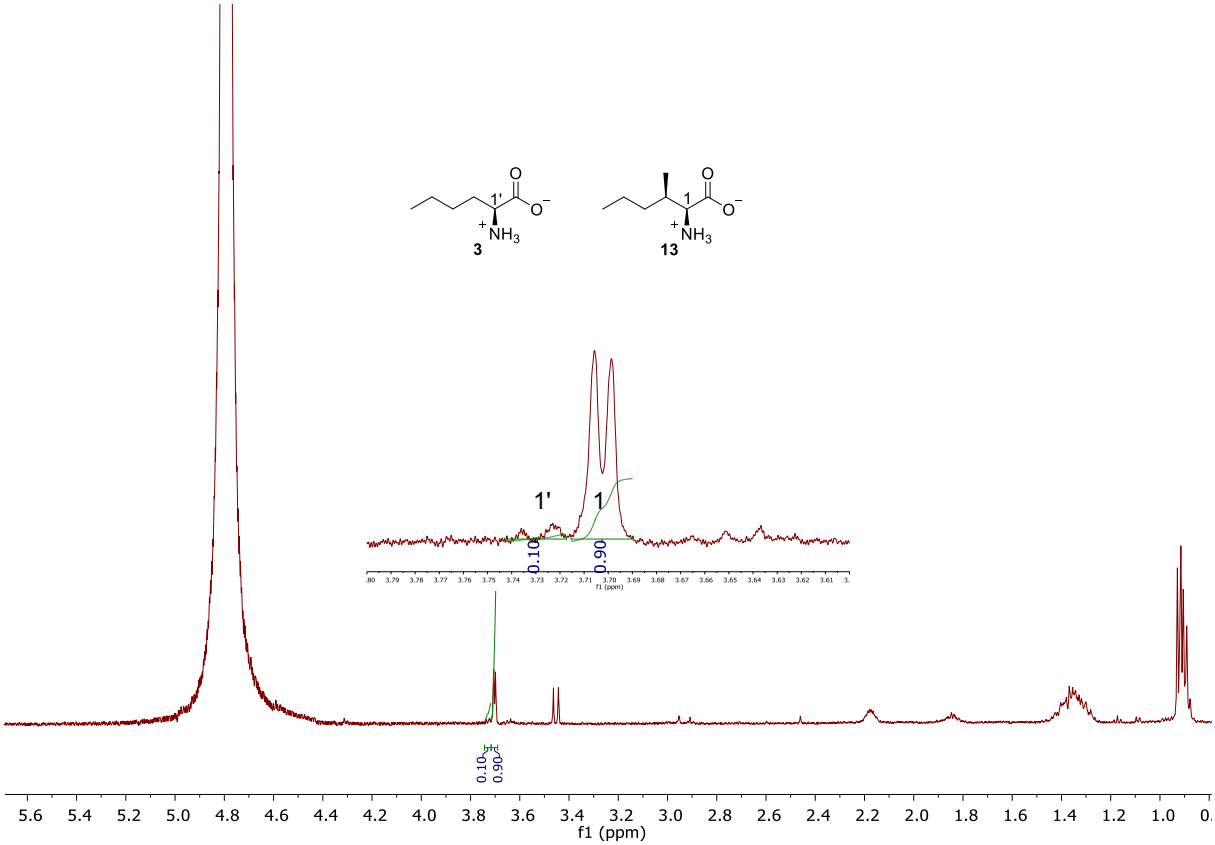
Supplementary Figure 5. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-norvaline (**1**) as substrate. Ratio was calculated based on integrals of methyl groups from both product and putative isomer (98:2).



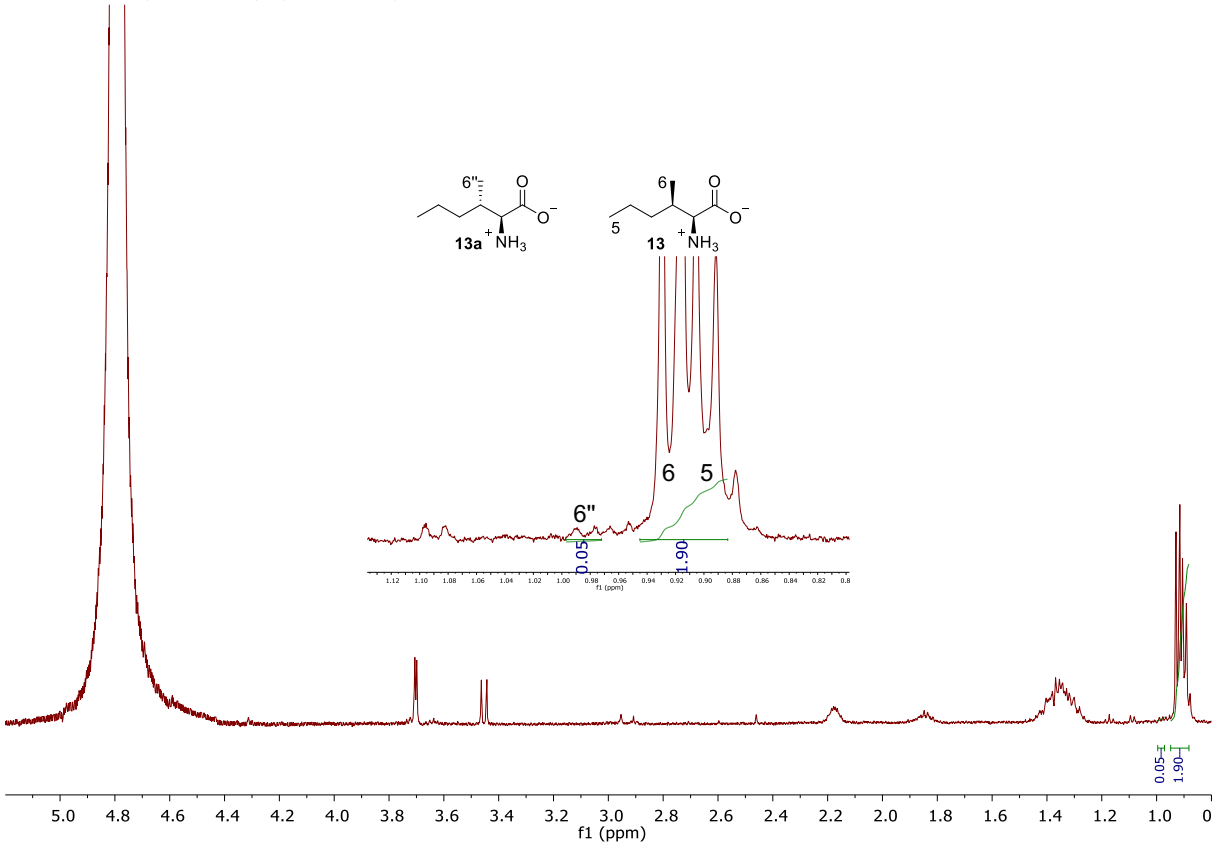
Supplementary Figure 6. Conversion of IlvE-SgvM cascade taking L-leucine (**2**) as substrate. Conversion was calculated based on integrals of α-H from both substrate and product. Conversion = 0.89/(0.11 + 0.89) x 100% = 89%.



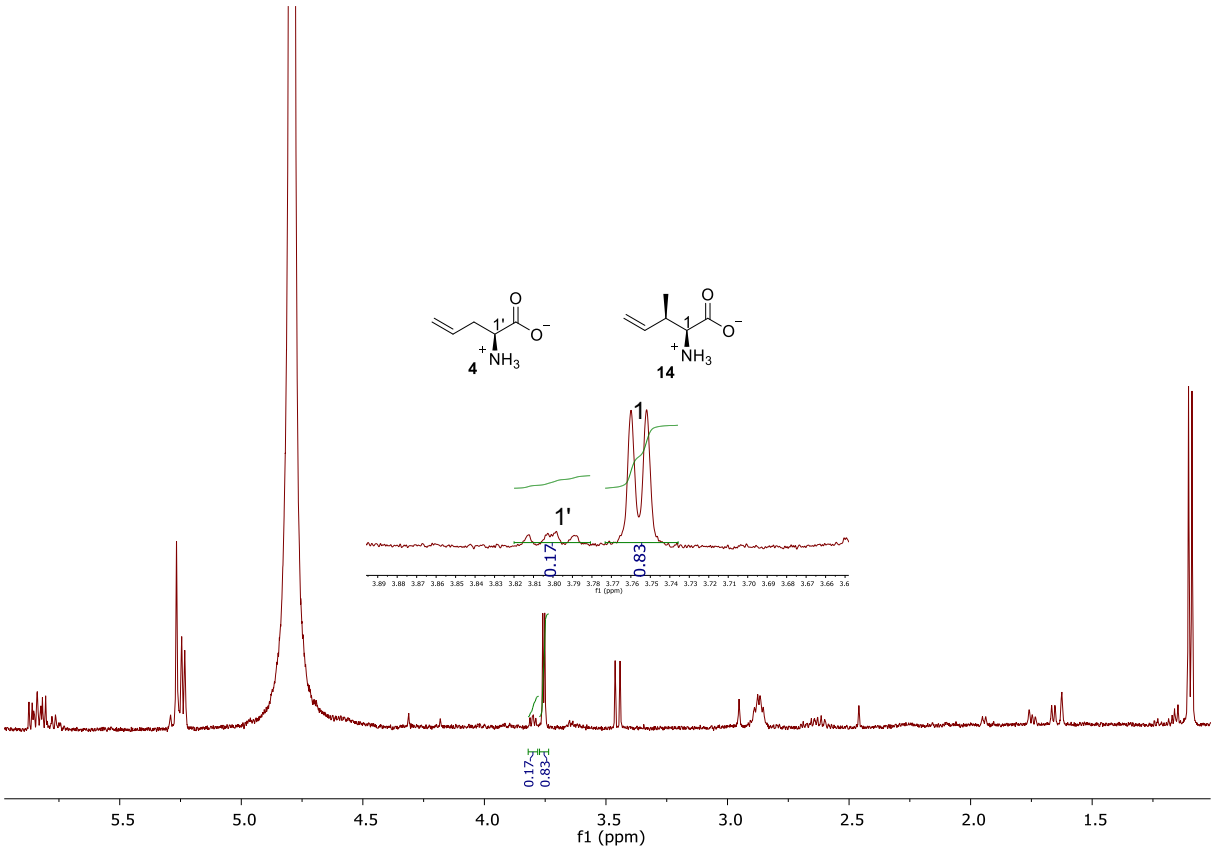
Supplementary Figure 7. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-leucine (**2**) as substrate. Ratio was calculated based on integrals of methyl groups from both product and putative isomer (99:1).



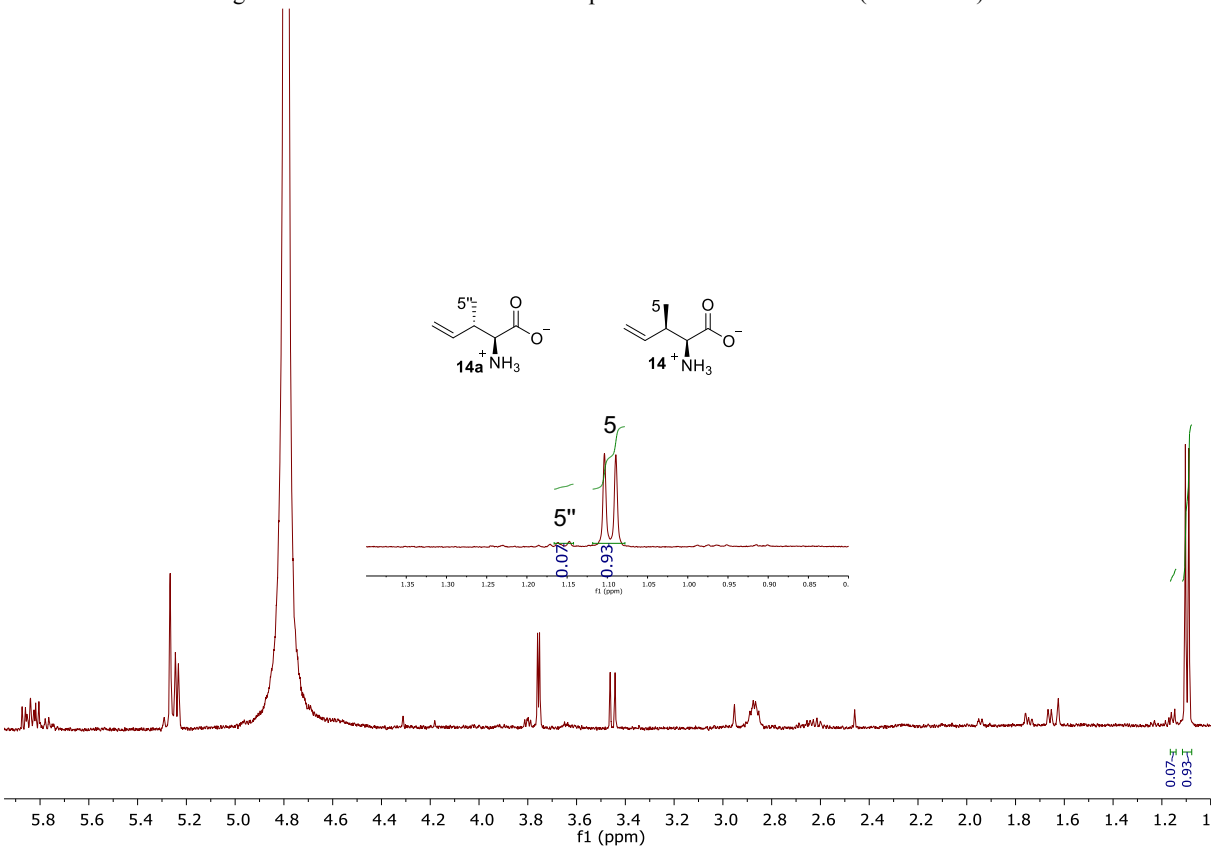
Supplementary Figure 8. Conversion of IlvE-SgvM cascade taking L-norleucine (**3**) as substrate. Conversion was calculated based on integrals of α-H from both substrate and product. Signal of α-H from **3** was partially overlapping with that from **13**. Conversion = (0.90 – 0.10) /(0.10 + 0.90) x 100% = 80 %.



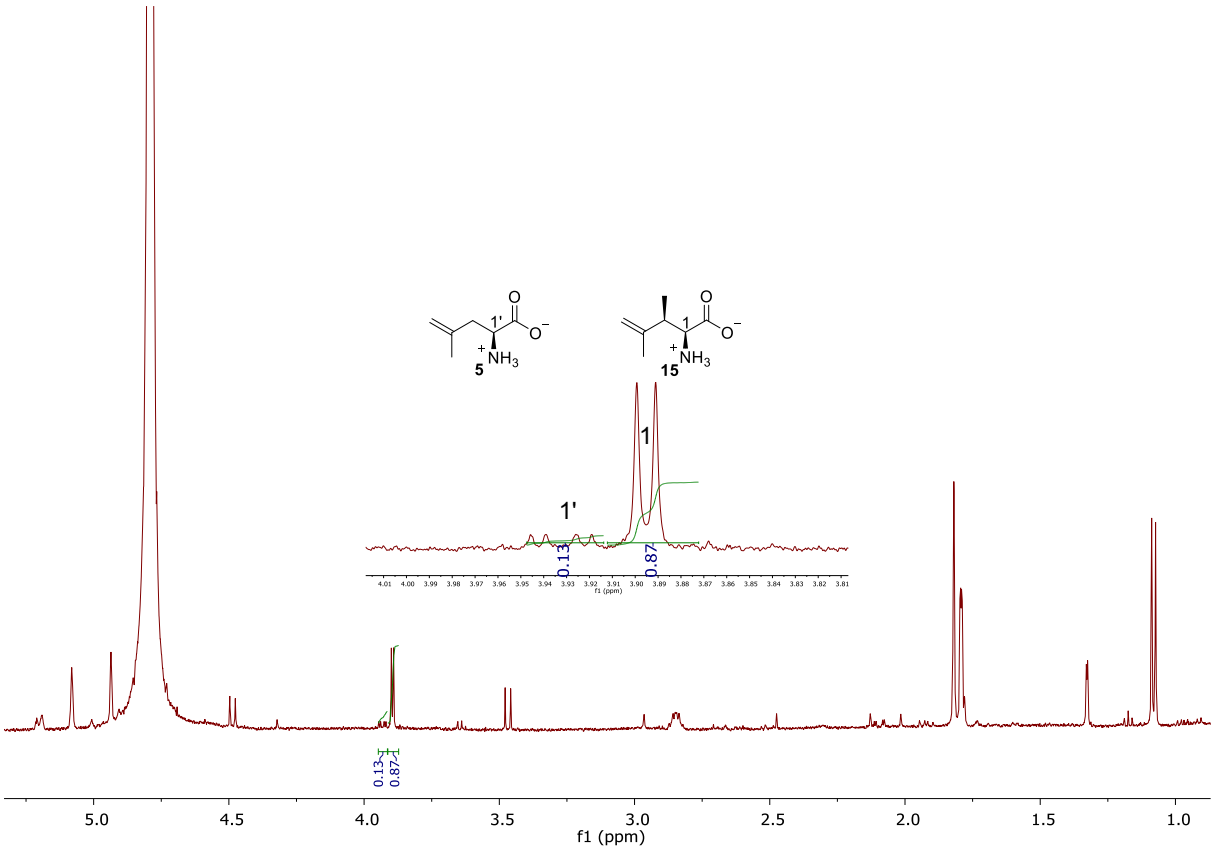
Supplementary Figure 9. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-norleucine (**3**) as substrate. Ratio was calculated based on integrals of methyl groups from both product and putative isomer (95:5).



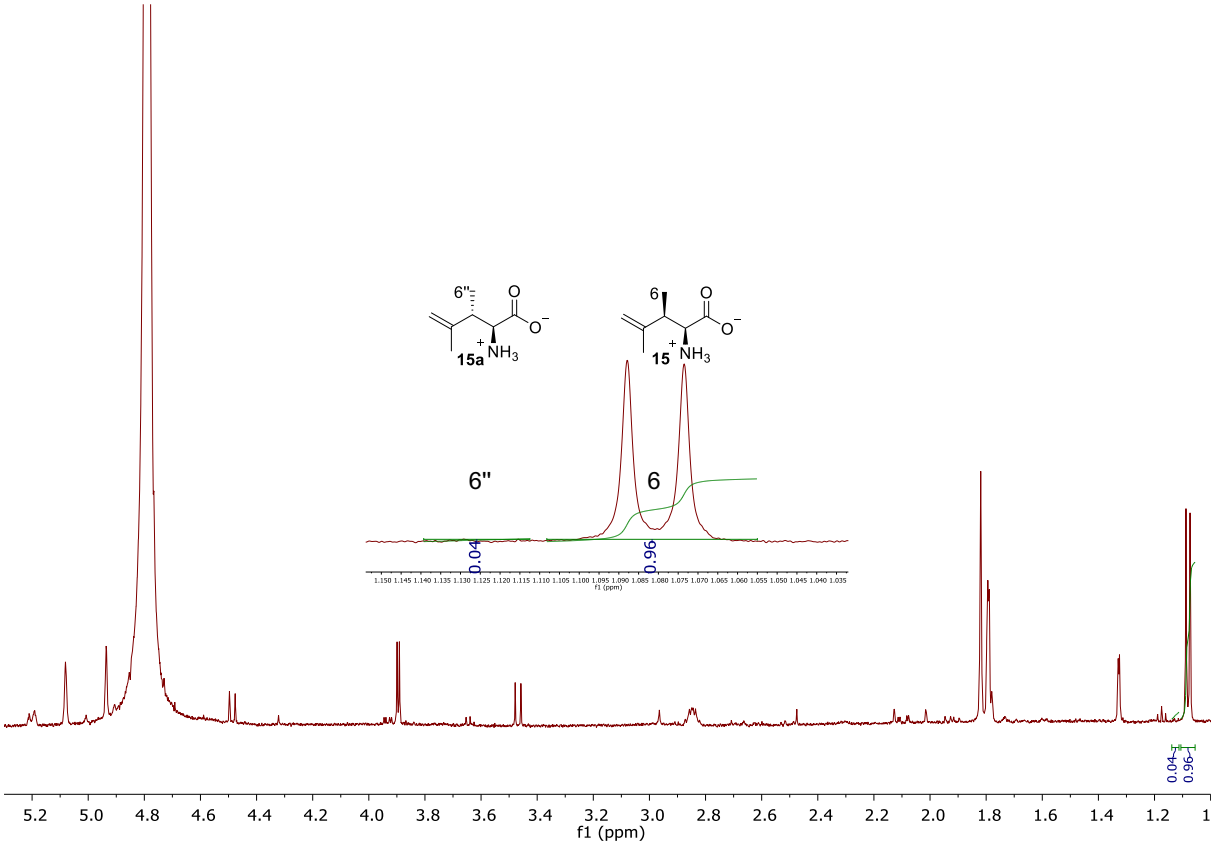
Supplementary Figure 10. Conversion of IlvE-SgvM cascade taking L-allylglycine (**4**) as substrate. Conversion was calculated based on integrals of α-H from both substrate and product. Conversion = 0.83/(0.17 + 0.83) x 100% = 83 %.



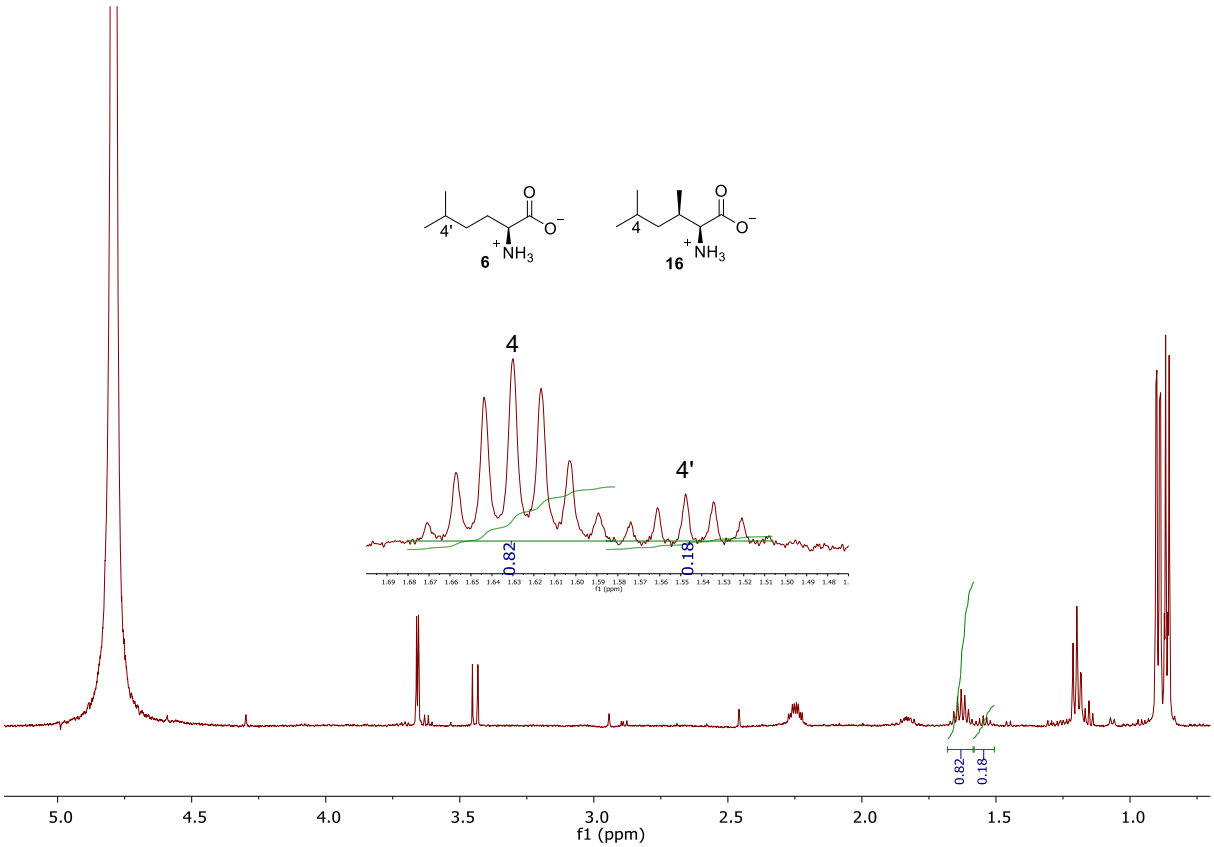
Supplementary Figure 11. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-allylglycine (**4**) as substrate. Ratio was calculated based on integrals of β-methyl groups from both product and putative isomer (93:7).



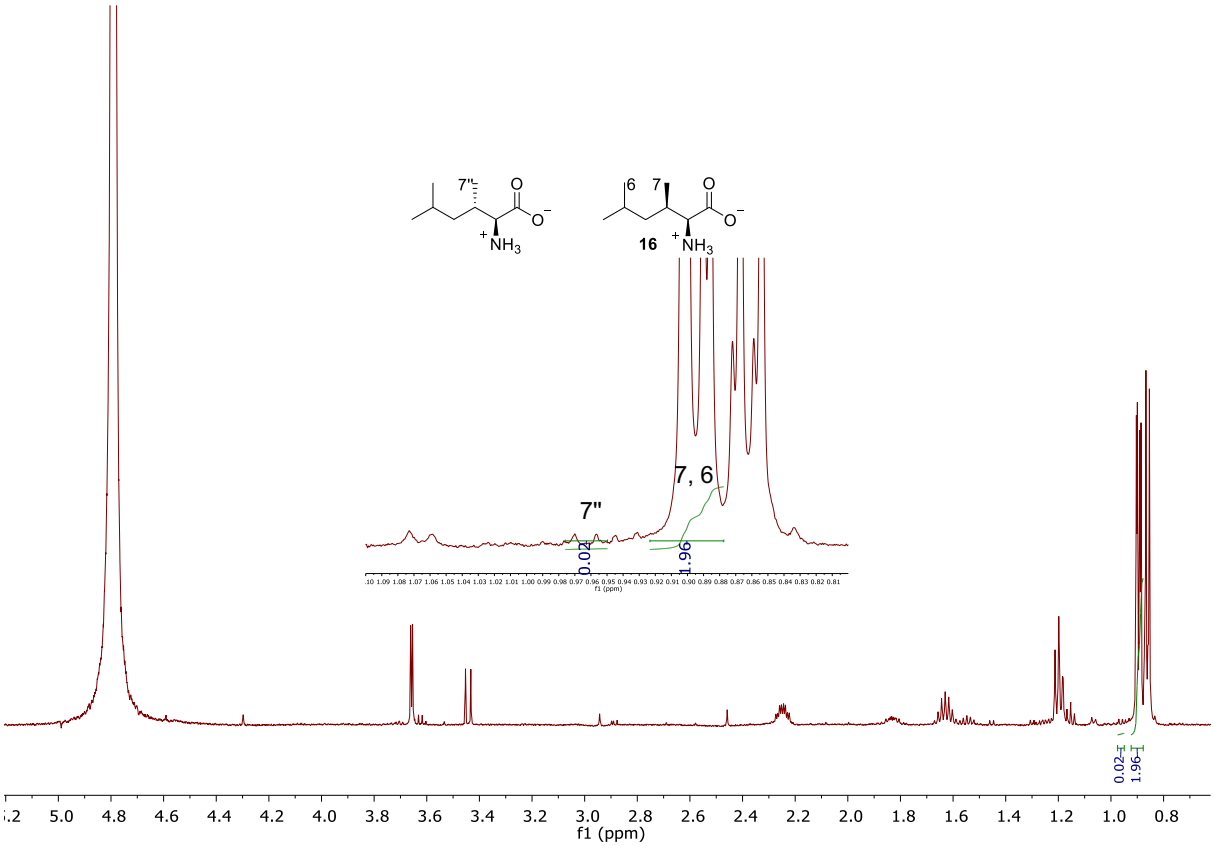
Supplementary Figure 12. Conversion of IlvE-SgvM cascade taking L-4,5-dehydroleucine (**5**) as substrate. Conversion was calculated based on integrals of α-H from both substrate and product. Conversion = 0.87/(0.13 + 0.87) x 100% = 87 %.



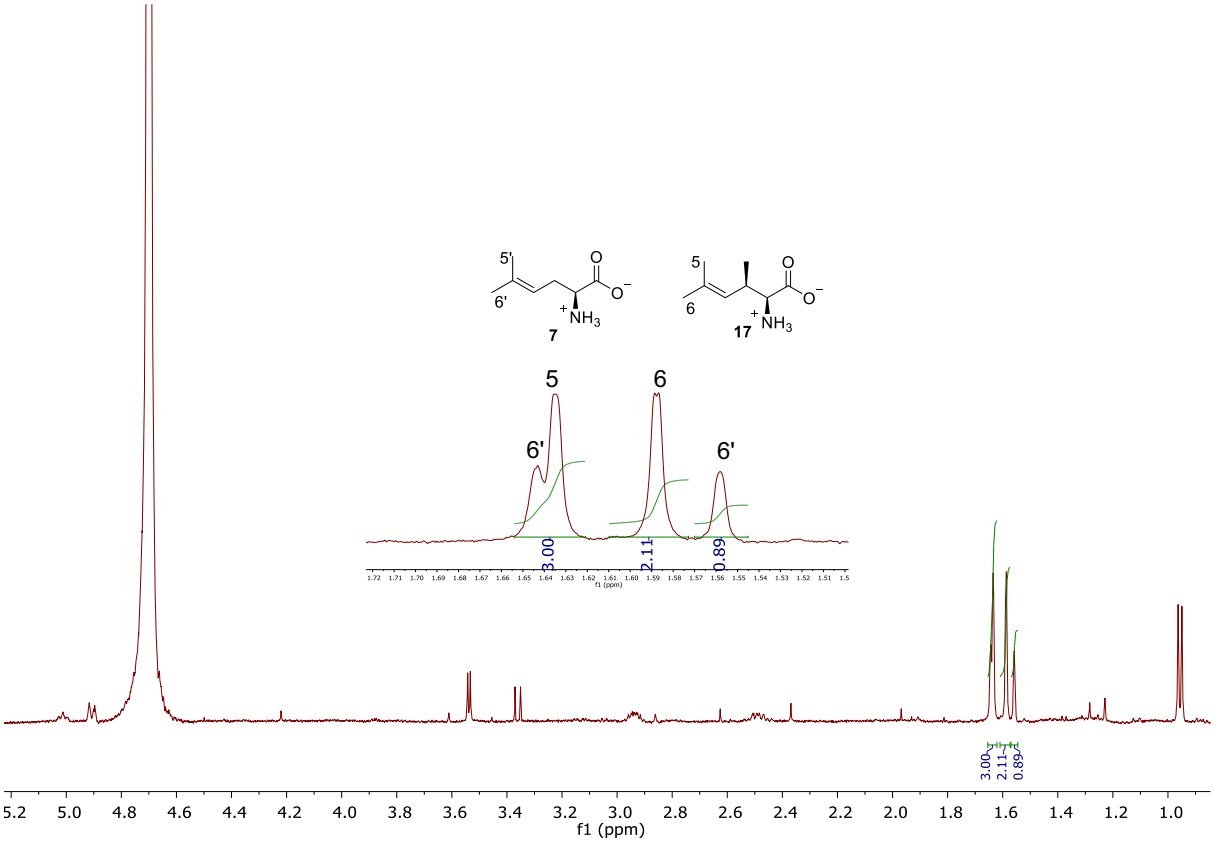
Supplementary Figure 13. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-4,5-dehydroleucine (**5**) as substrate. Ratio was calculated based on integrals of β-methyl groups from both product and putative isomer (96:4).



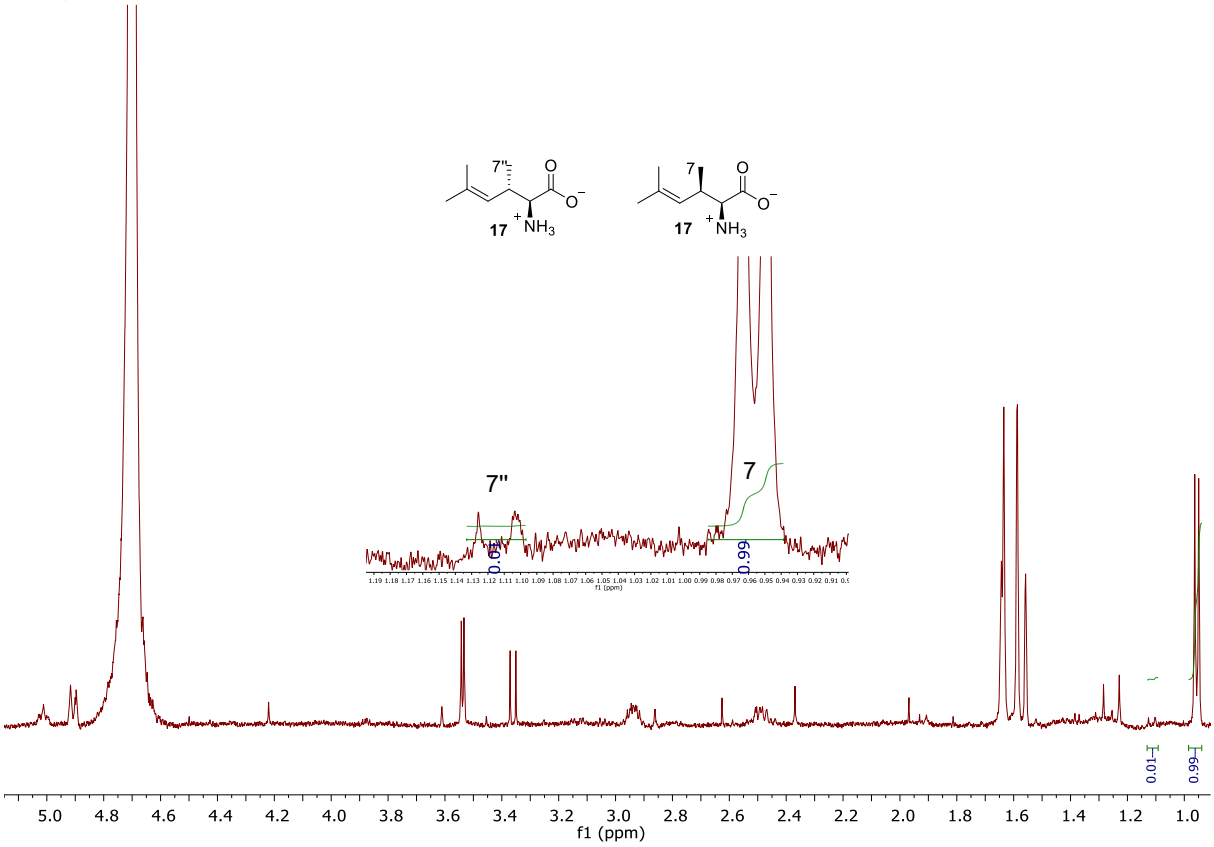
Supplementary Figure 14. Conversion of IlvE-SgvM cascade taking L-2-amino-5-methyl hexanoic acid (**6**) as substrate. Conversion was calculated based on integrals of ε-H from both substrate and product. Conversion = 0.82/(0.18 + 0.82) x 100% = 82%.



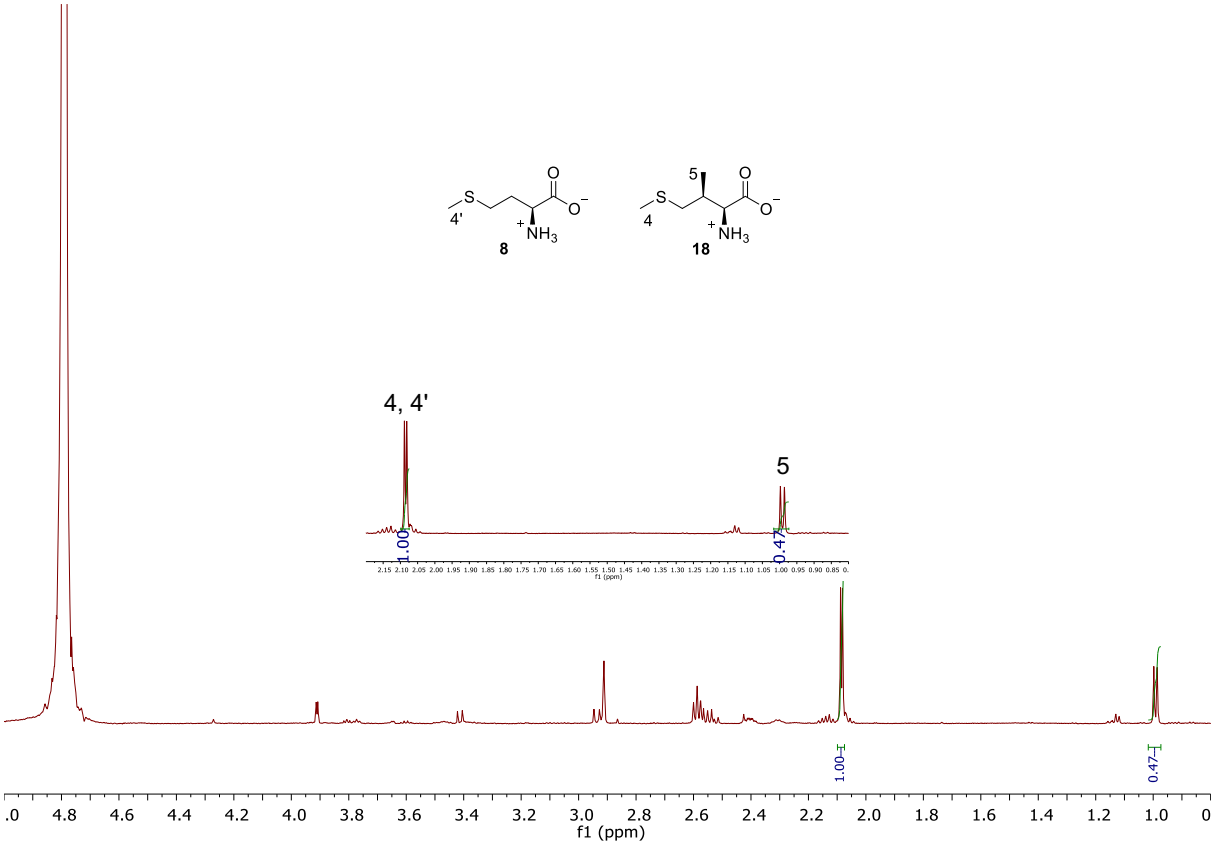
Supplementary Figure 15. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-2-amino-5-methyl hexanoic acid (**6**) as substrate. Ratio was calculated based on integrals of methyl groups from both product and putative isomer (98:2).



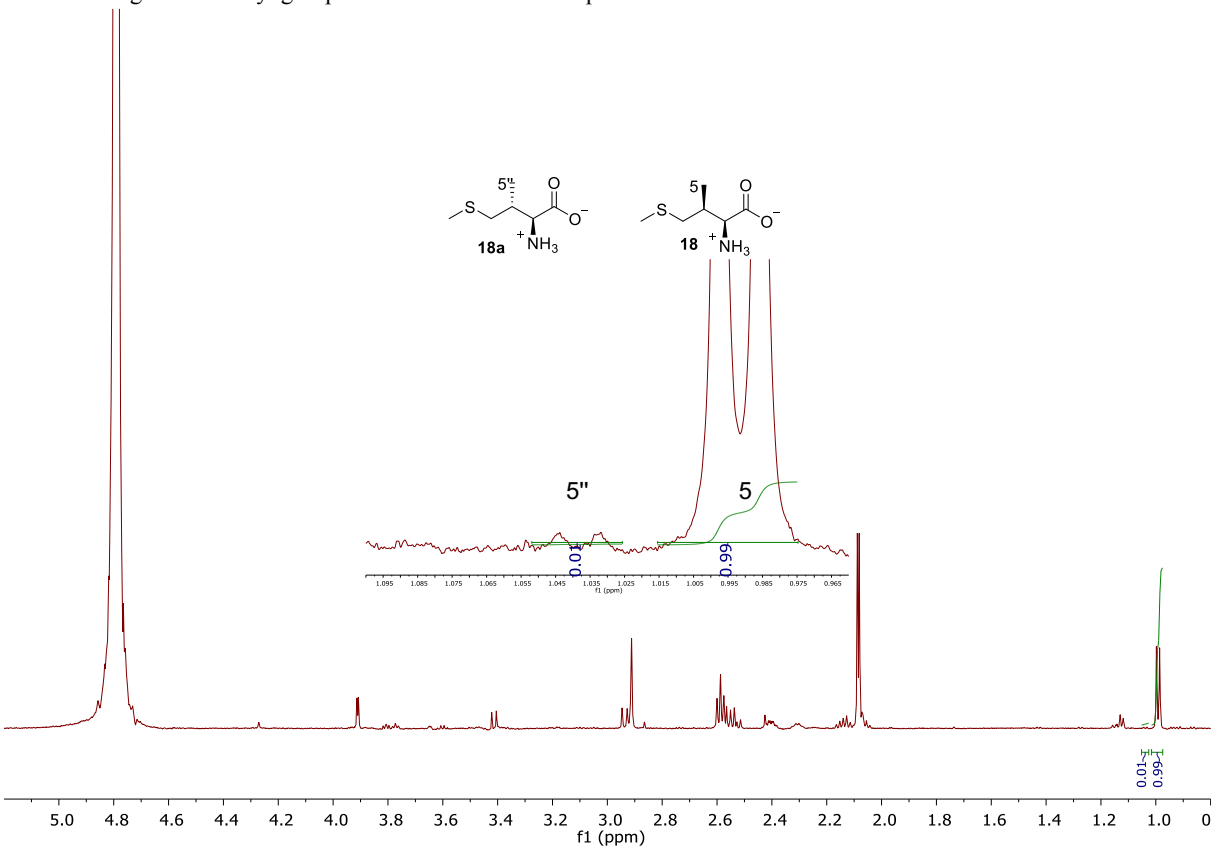
Supplementary Figure 16. Conversion of IlvE-SgvM cascade taking L-2-amino-5-methyl hexenoic acid (**7**) as substrate. Conversion was calculated based on integrals of allylic methyl groups from both substrate and product. Conversion = 2.11/(2.11 + 0.89) x 100% = 70 %.



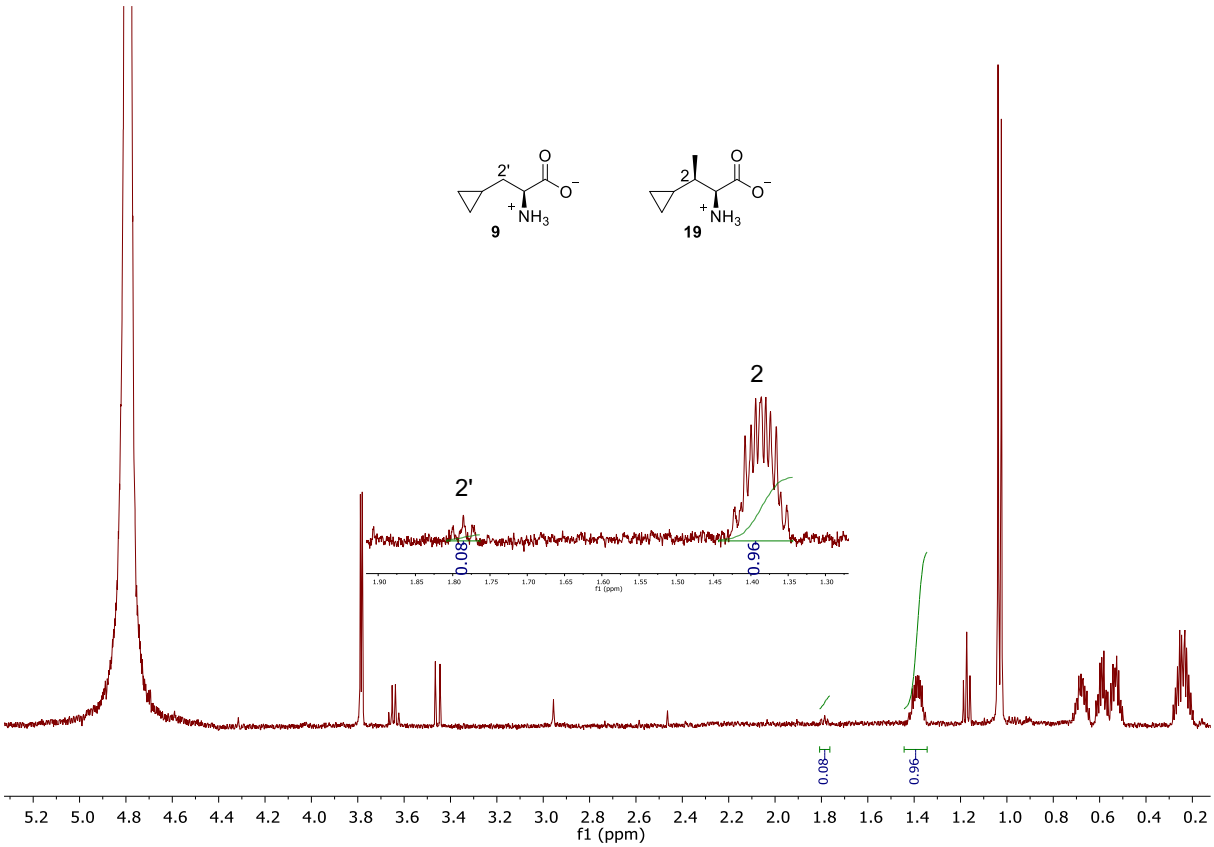
Supplementary Figure 17. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-2-amino-5-methyl hexenoic acid (**7**) as substrate. Ratio was calculated based on integrals of β-methyl groups from both product and putative isomer (99:1).



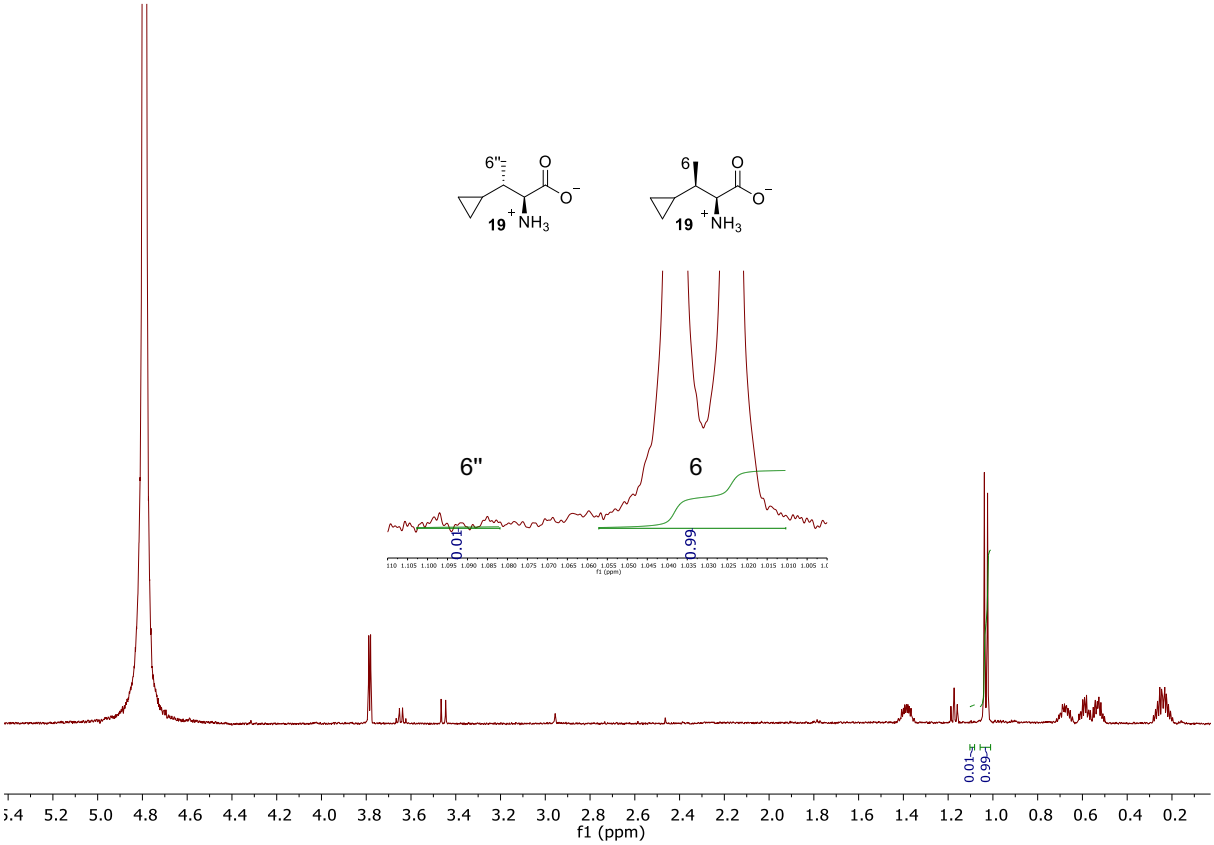
Supplementary Figure 18. Conversion of IlvE-SgvM cascade taking L-methionine (**8**) as substrate. Conversion was calculated based on integrals of methyl groups from both substrate and product. Conversion = 0.47/1.00 x 100% = 47%.



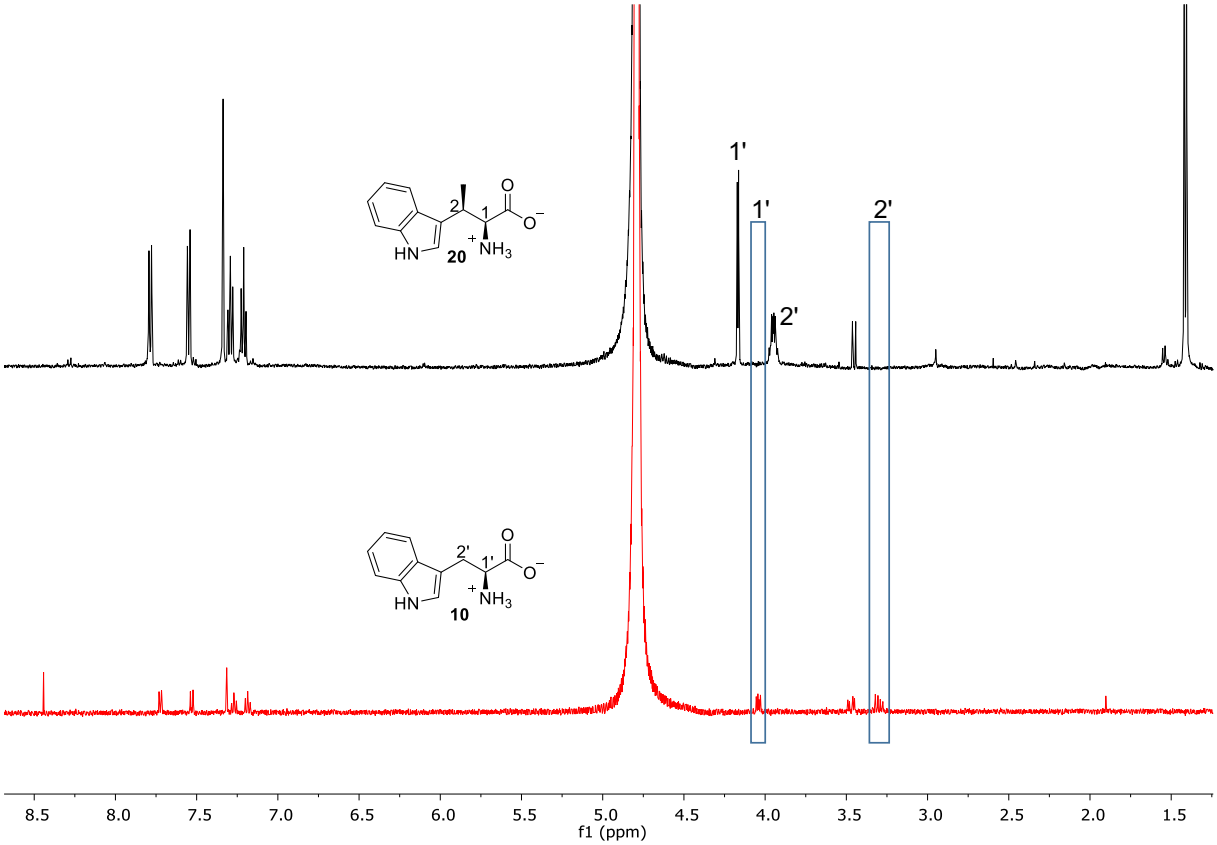
Supplementary Figure 19. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-methionine (**8**) as substrate. Ratio was calculated based on integrals of β-methyl groups from both product and putative isomer (99:1).



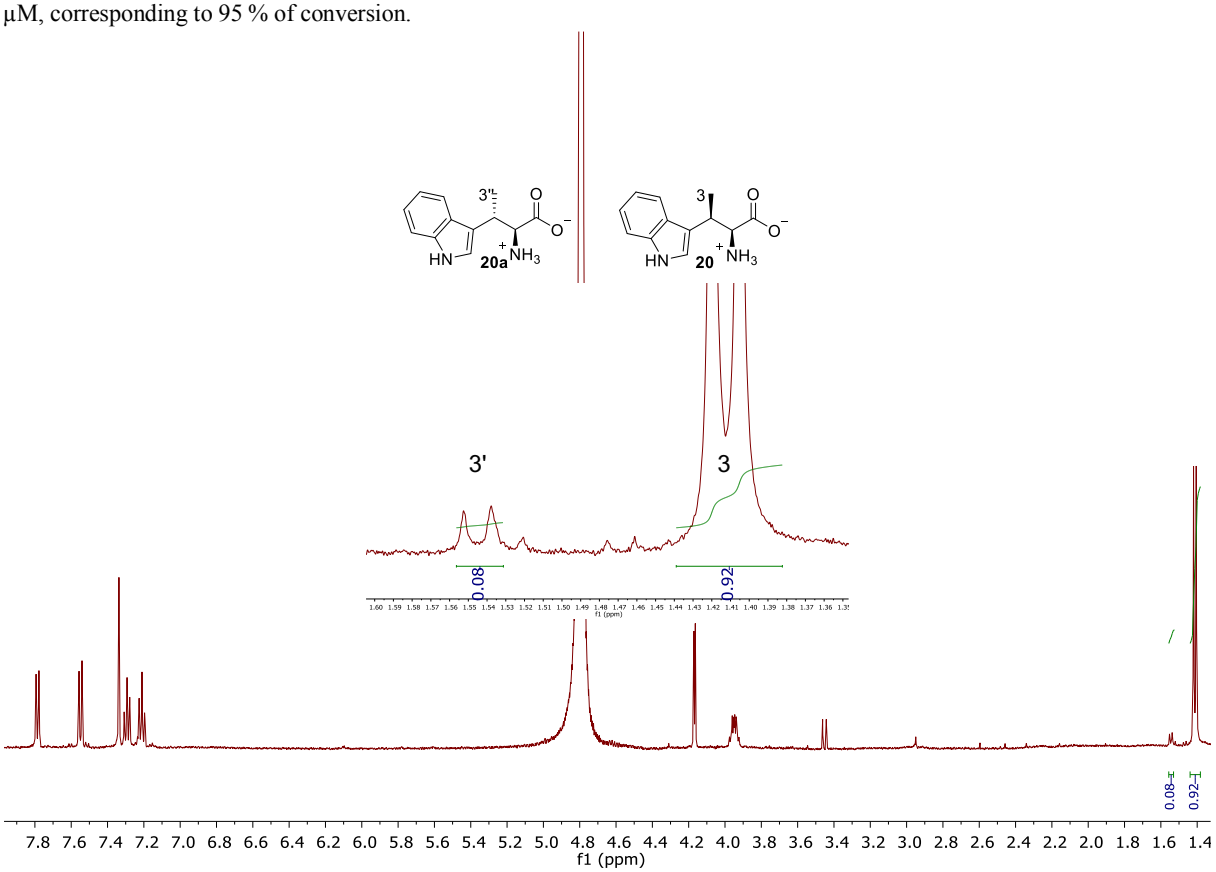
Supplementary Figure 20. Conversion of IlvE-SgvM cascade taking L-cyclopropylalanine (**9**) as substrate. Conversion was calculated based on integrals of β-H from both substrate and product. Conversion = 0.96/(0.96 + 0.08/2) x 100% = 96 %.



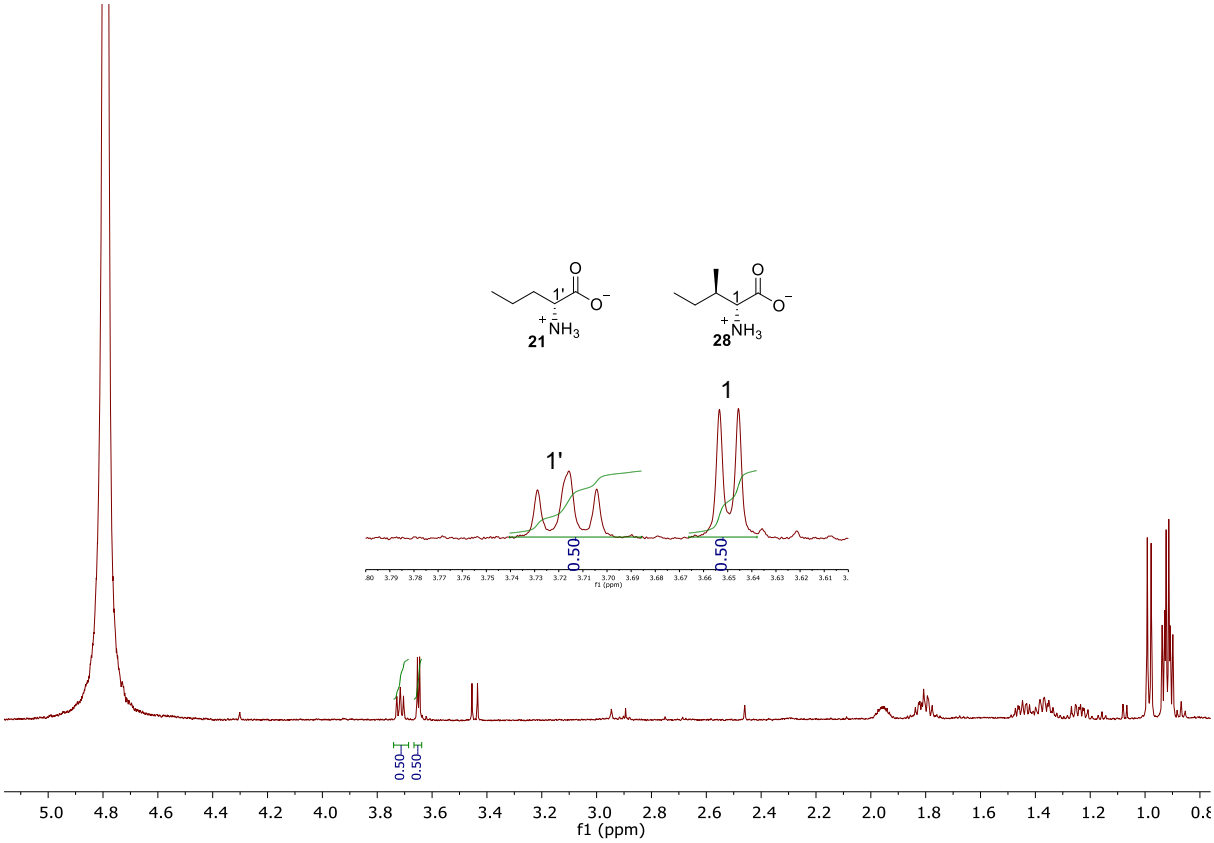
Supplementary Figure 21. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-cyclopropylalanine (**9**) as substrate. Ratio was calculated based on integrals of β-methyl groups from both product and putative isomer (99:1).



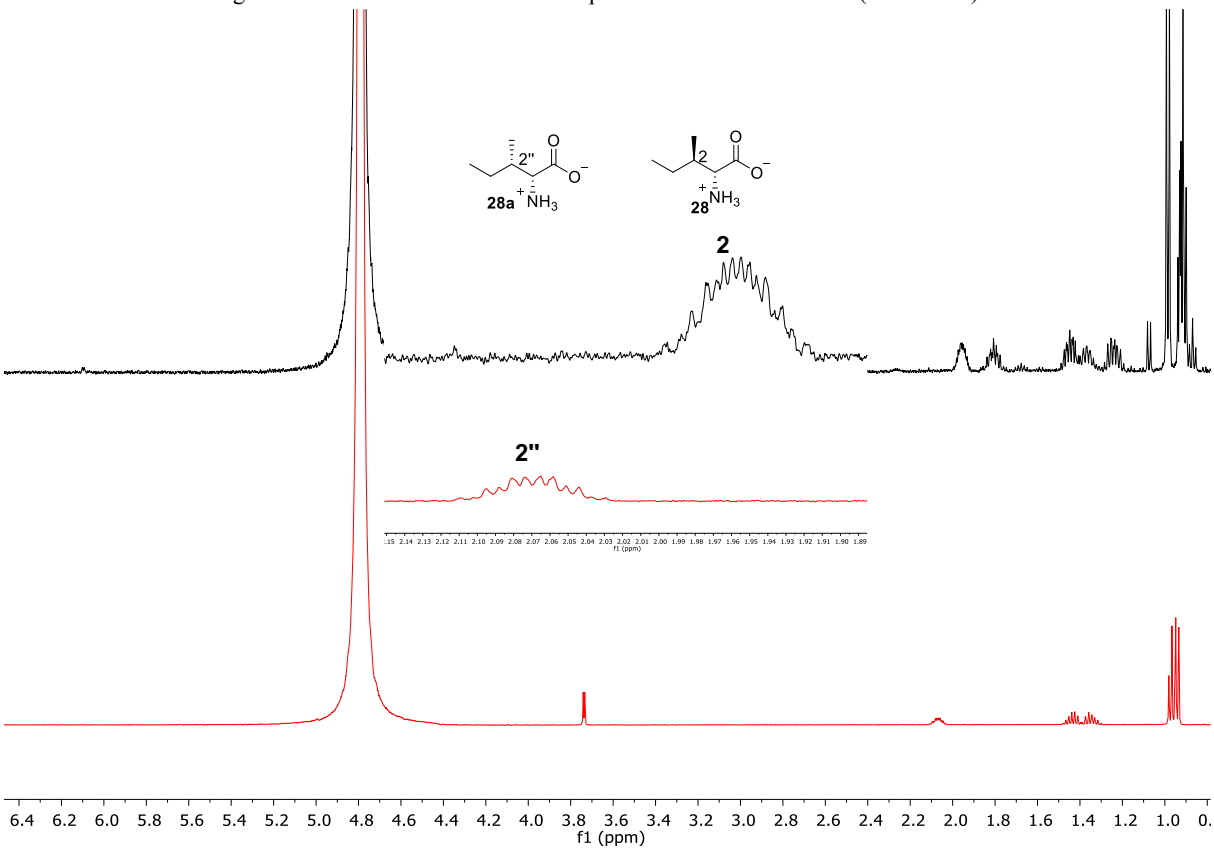
Supplementary Figure 22. Conversion of MarG-MarI cascade taking L-tryptophan (**10**) as substrate. Conversion was determined over 95 %, because no substrate was observed in reaction mixture (**Top**) while resonances was evidently observed in control experiment with 100 µM substrate (**Bottom**). Therefore, residue substrate within reaction mixture is lower than 100 µM, corresponding to 95 % of conversion.



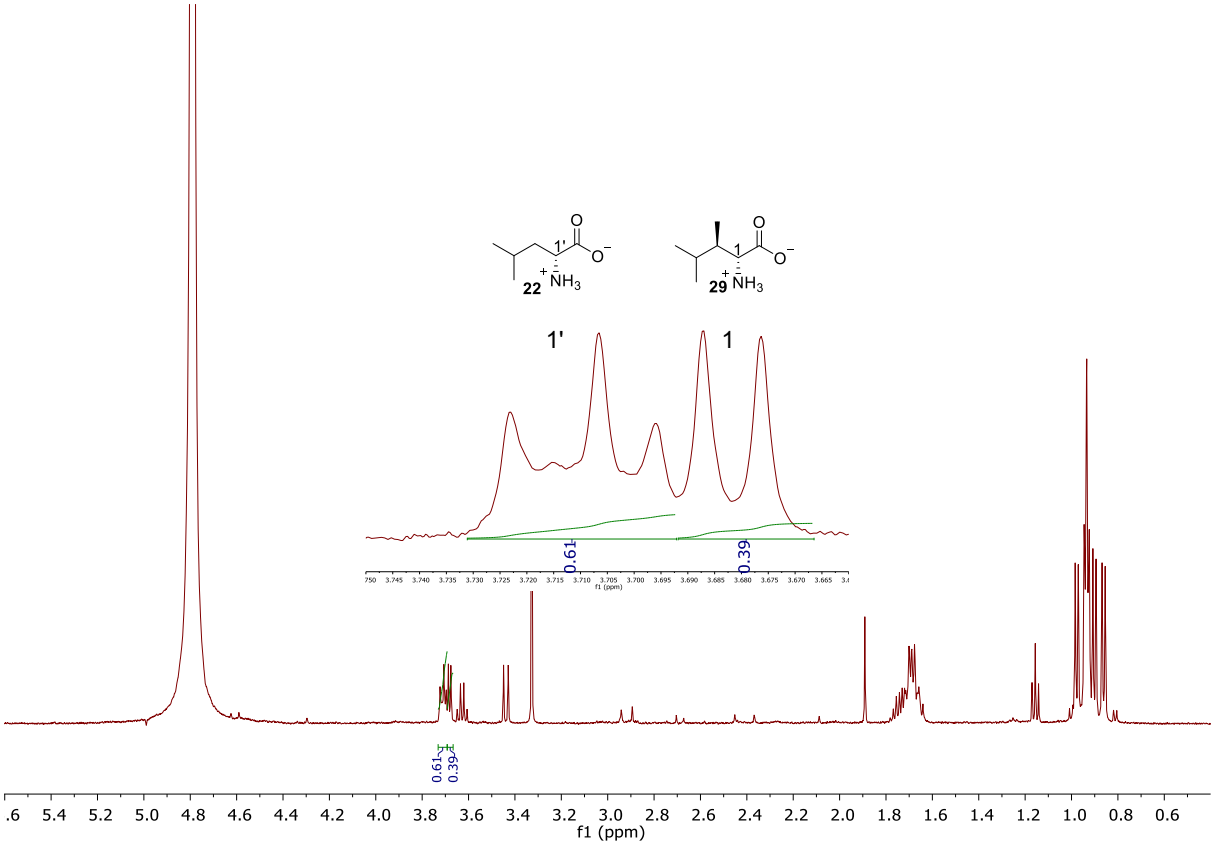
Supplementary Figure 23. 3R:3S ratio of product of IlvE-SgvM cascade reaction using L-tryptophan (**10**) as substrate. Ratio was calculated based on integrals of β-methyl groups from both both product and putative isomer (92:8).



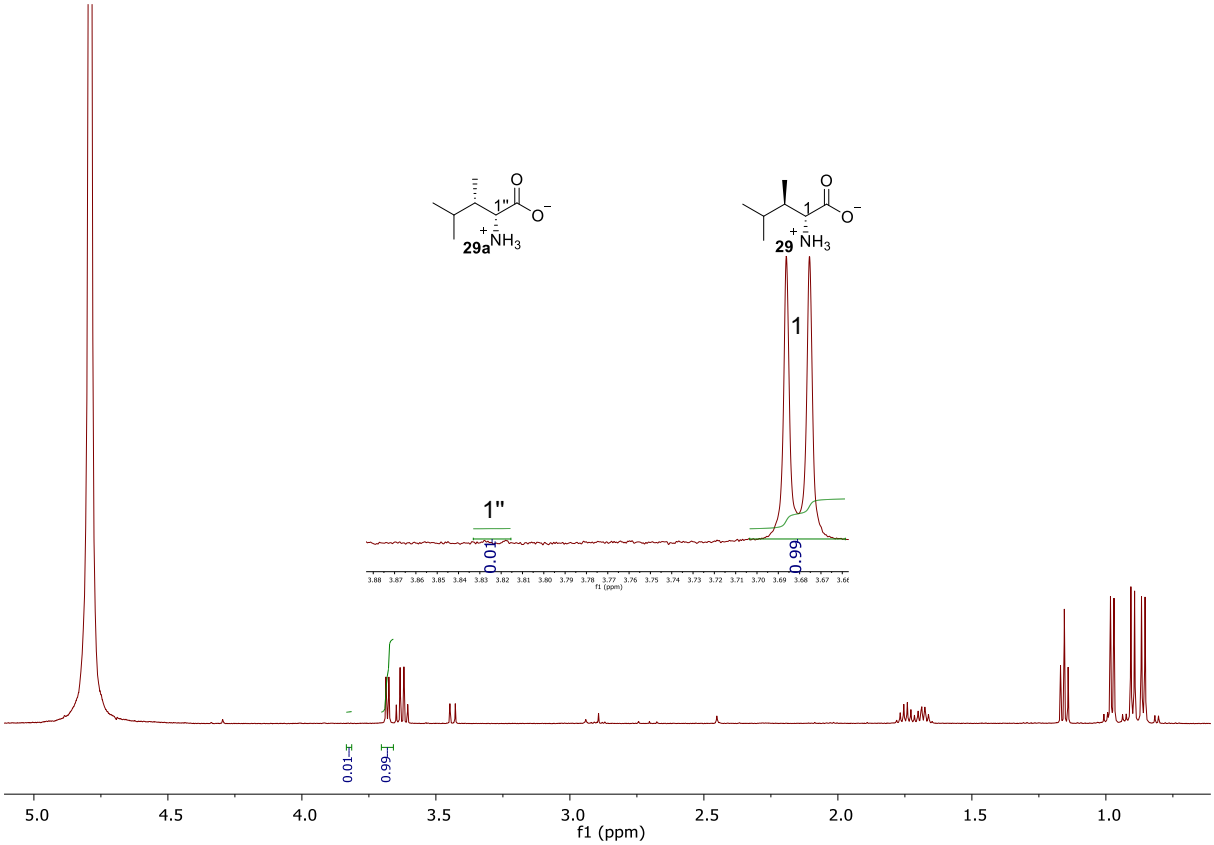
Supplementary Figure 24. Conversion of D-TA-SgvM cascade taking D-norvaline (**21**) as substrate Conversion was calculated based on integrals of α-H from both substrate and product. Conversion = 0.50/(0.50 +0.50) x 100% = 50 %



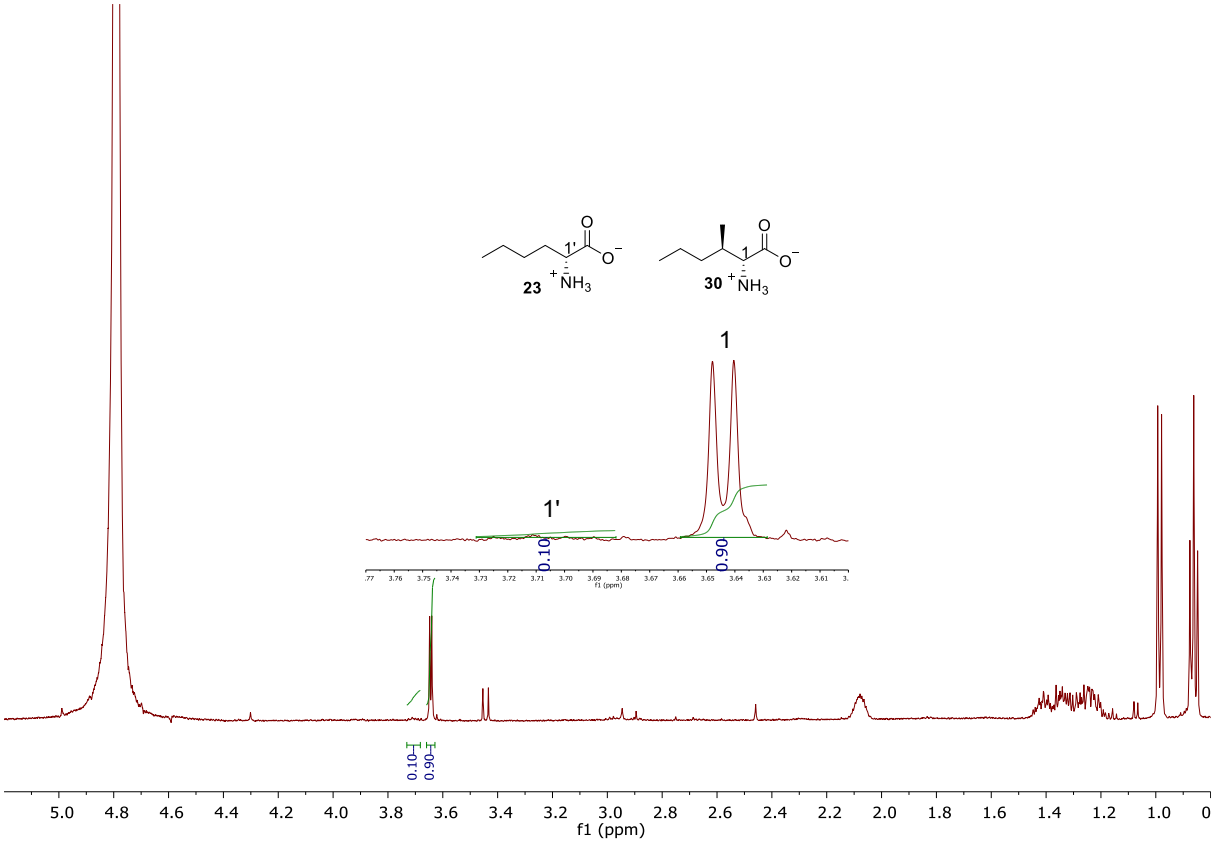
Supplementary Figure 25. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-norvaline (**21**) as substrate. No signal from D-*allo*-isoleucine (**28a**) was observed. indicating the concentration of is below 40 µM. Therefore, the ratio is over >98:2.



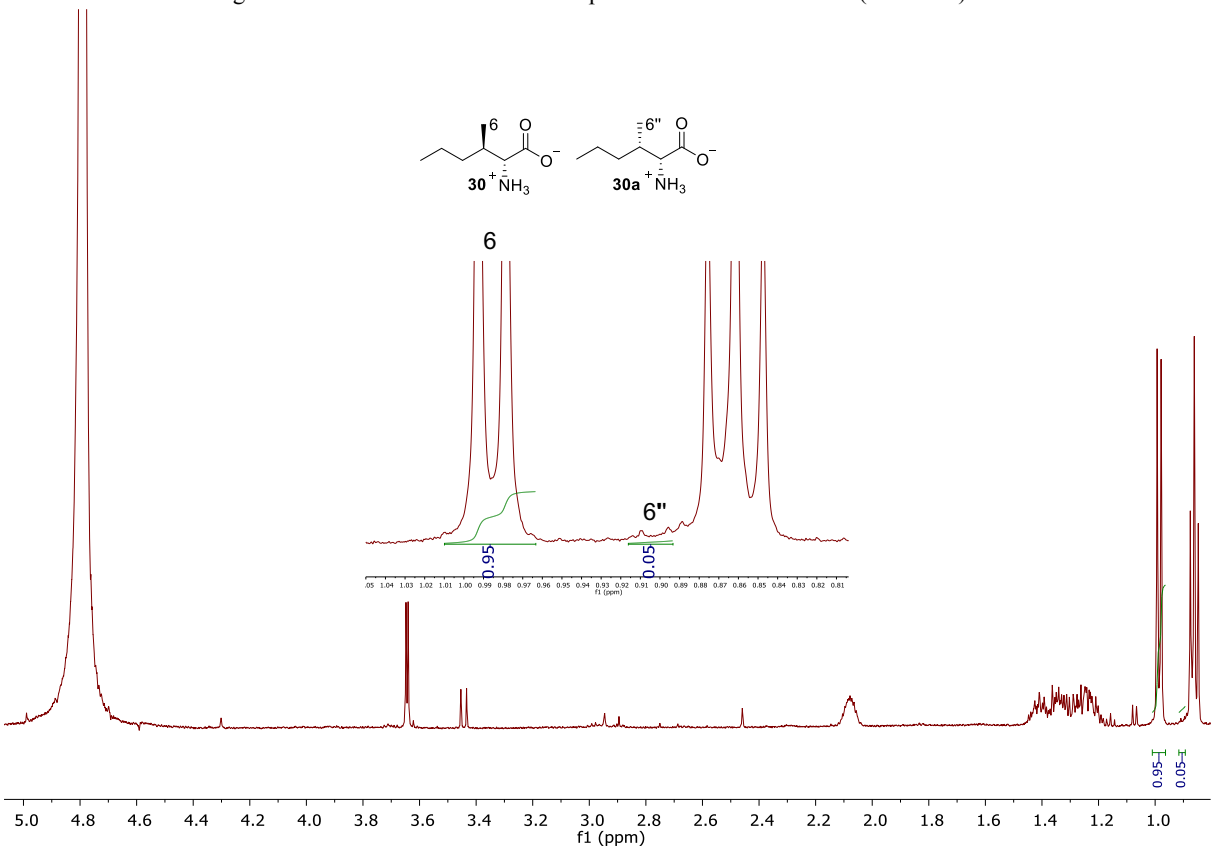
Supplementary Figure 26. Conversion of D-TA-SgvM cascade taking D-leucine (**22**) as substrate Conversion was calculated based on integrals of α-H from both substrate and product. Conversion = 0.39/(0.39 +0.61) x 100% = 39 %



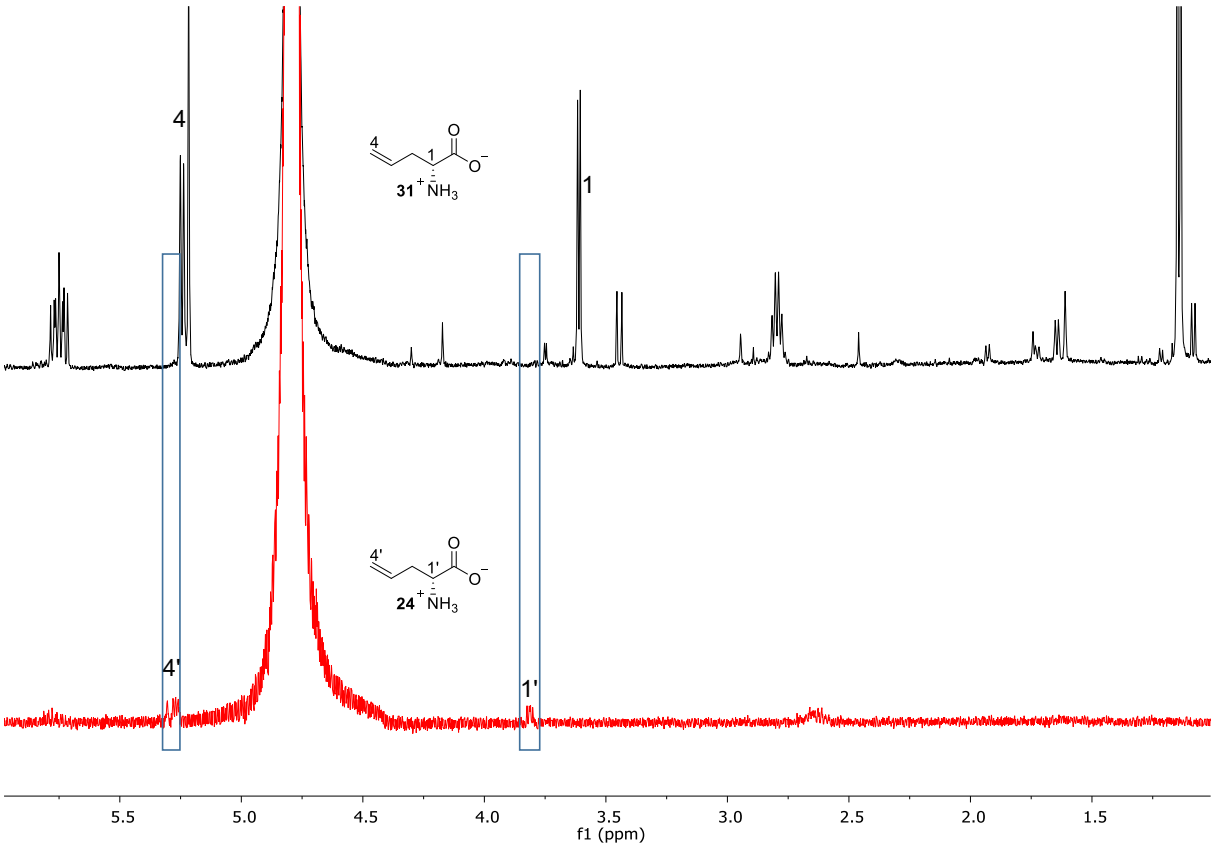
Supplementary Figure 27. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-leucine (**22**) as substrate. Ratio was calculated based on integrals of α-H from both product and putative isomer (99:1).



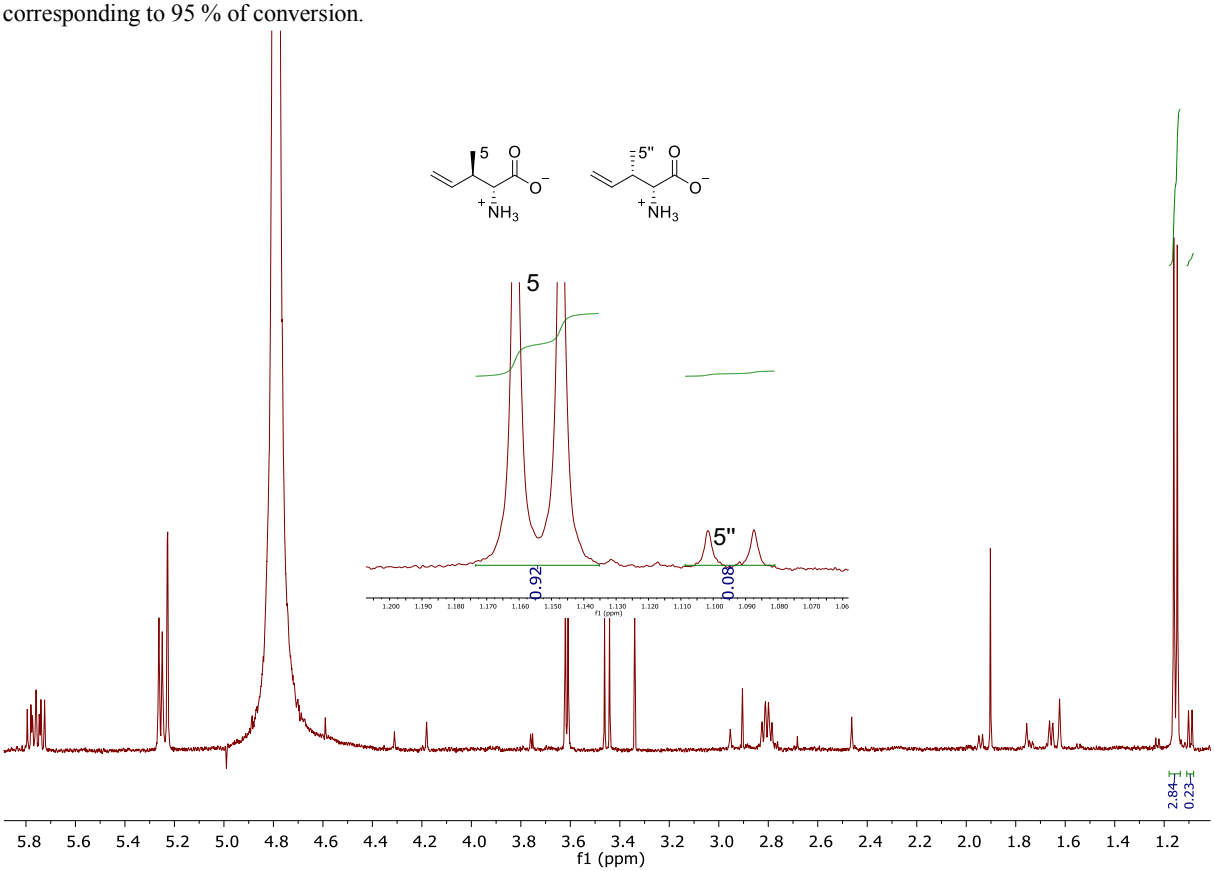
Supplementary Figure 28. Conversion of D-TA-SgvM cascade taking D-norleucine (**23**) as substrate Conversion was calculated based on integrals of α-H from both substrate and product. Conversion = 0.90/(0.9 +0.10) x 100% = 90 %



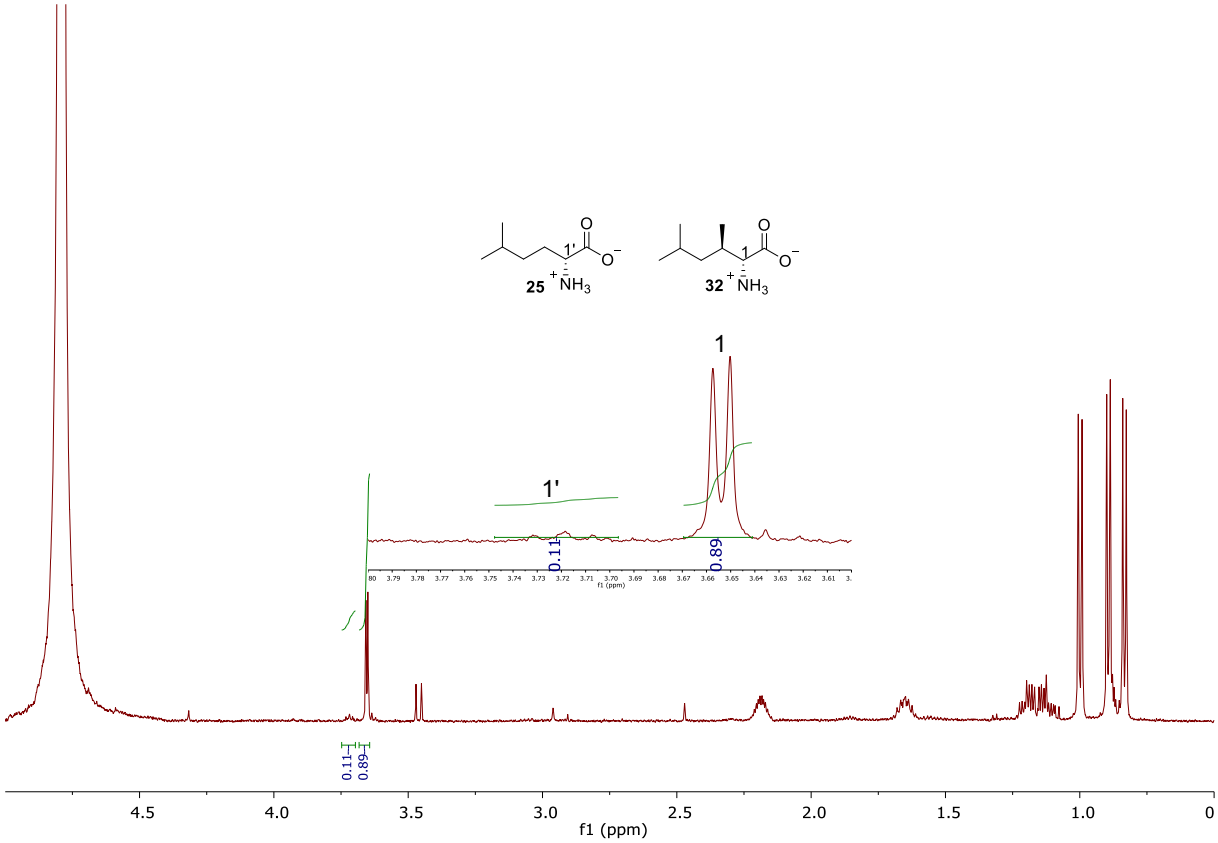
Supplementary Figure 29. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-norleucine (**23**) as substrate. Ratio was calculated based on integrals of β-methyl group from both product and putative isomer (95:5).



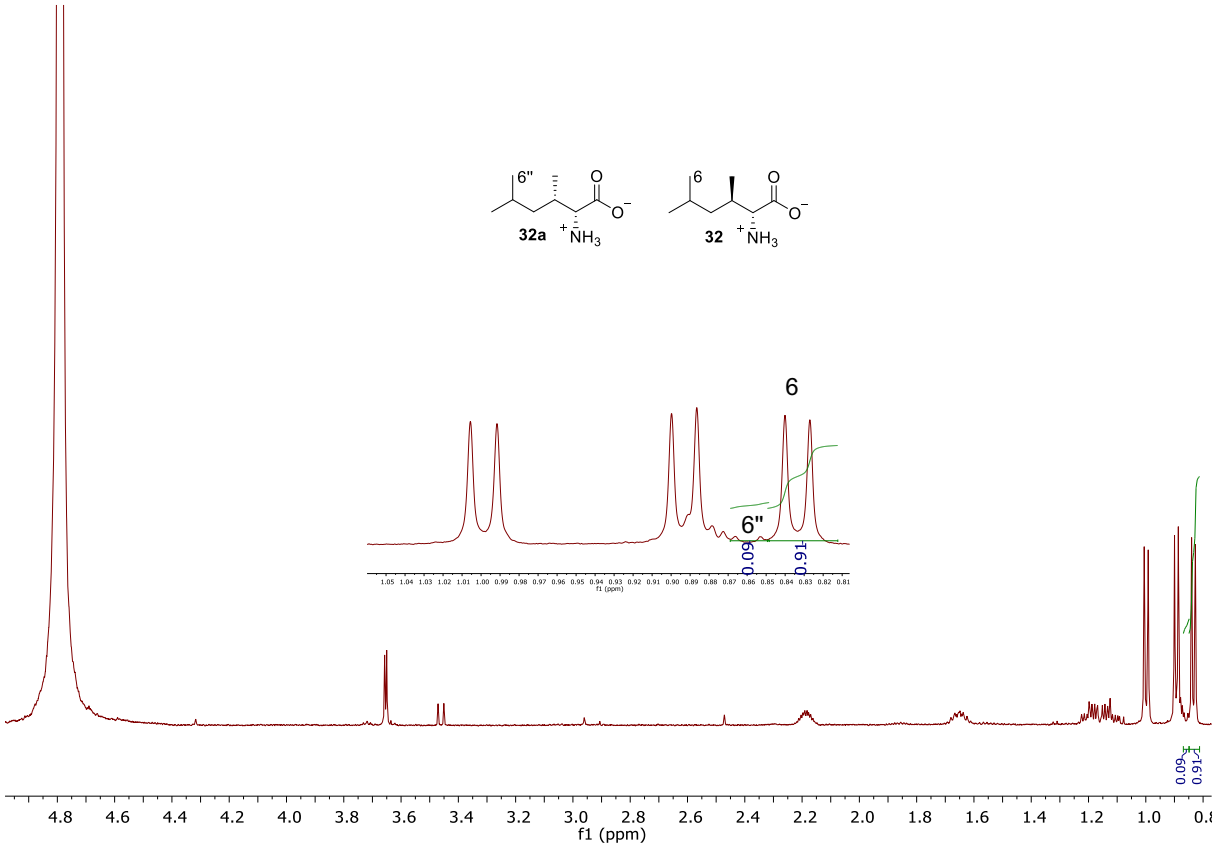
Supplementary Figure 30. Conversion of D-TA-SgvM cascade taking D-allylglycine (**24**). Conversion was determined over 95 %, because no substrate was observed in reaction mixture (**Top**) while resonances was evidently observed in control experiment with 100 µM substrate (**Bottom**). Therefore, residue substrate within reaction mixture is lower than 100 µM, corresponding to 95 % of conversion.



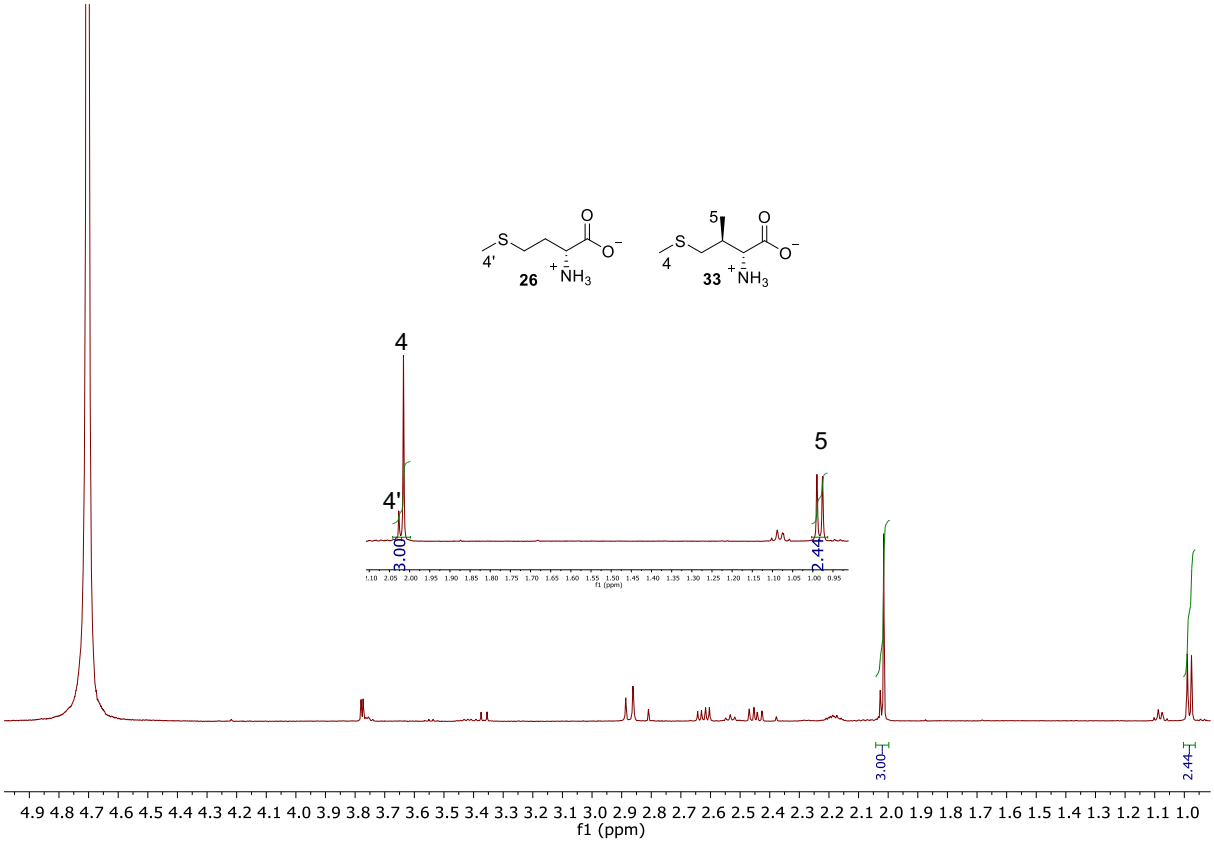
Supplementary Figure 31. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-allylglycine (**24**) as substrate. Ratio was calculated based on integrals of β-methyl group from both product and putative isomer (92:8).



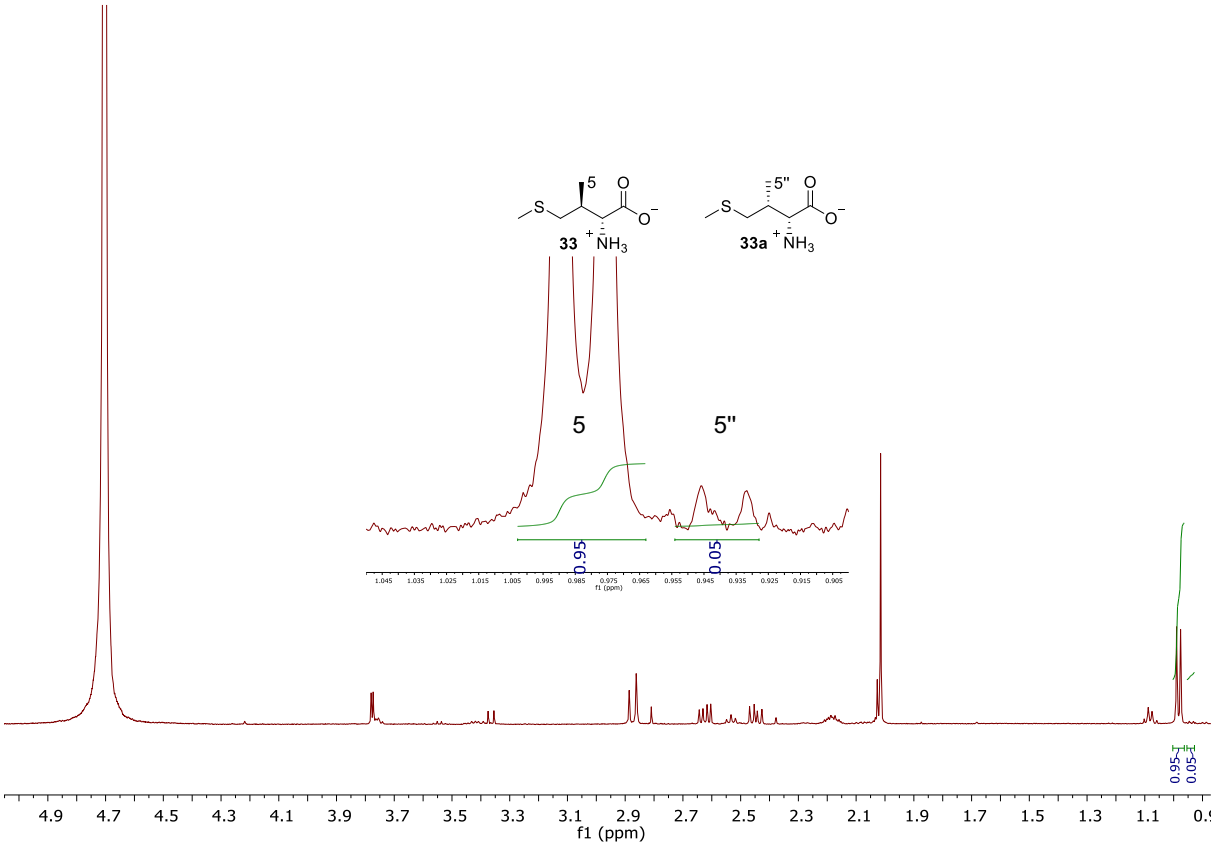
Supplementary Figure 32. Conversion of D-TA-SgvM cascade taking D-2-amino-5-methyl hexanoic acid (**25**) as substrate Conversion was calculated based on integrals of α-H from both substrate and product. Conversion = 0.89/(0.89 +0.11) x 100% = 89 %



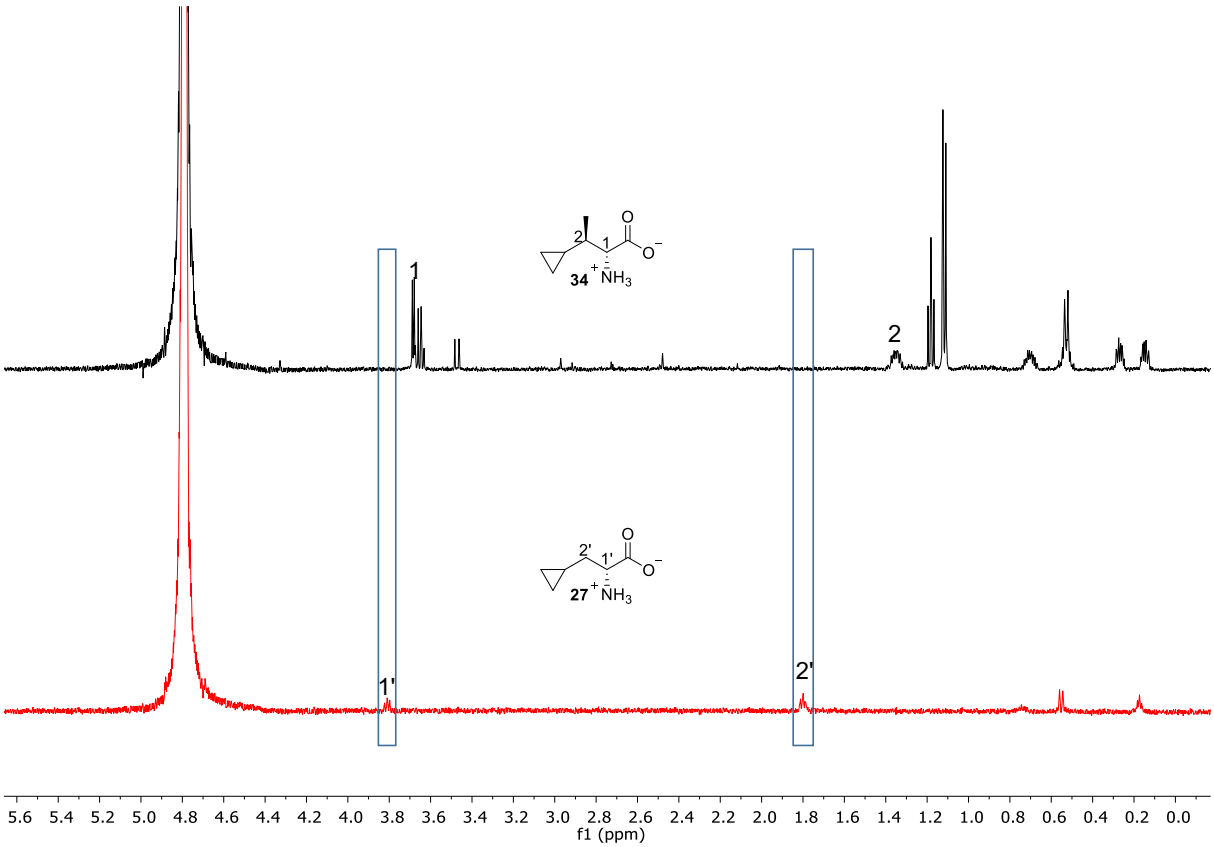
Supplementary Figure 33. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-2-amino-5-methyl hexanoic acid (**25**) as substrate. Ratio was calculated based on integrals of ε-methyl group from both product and putative isomer (91:9).



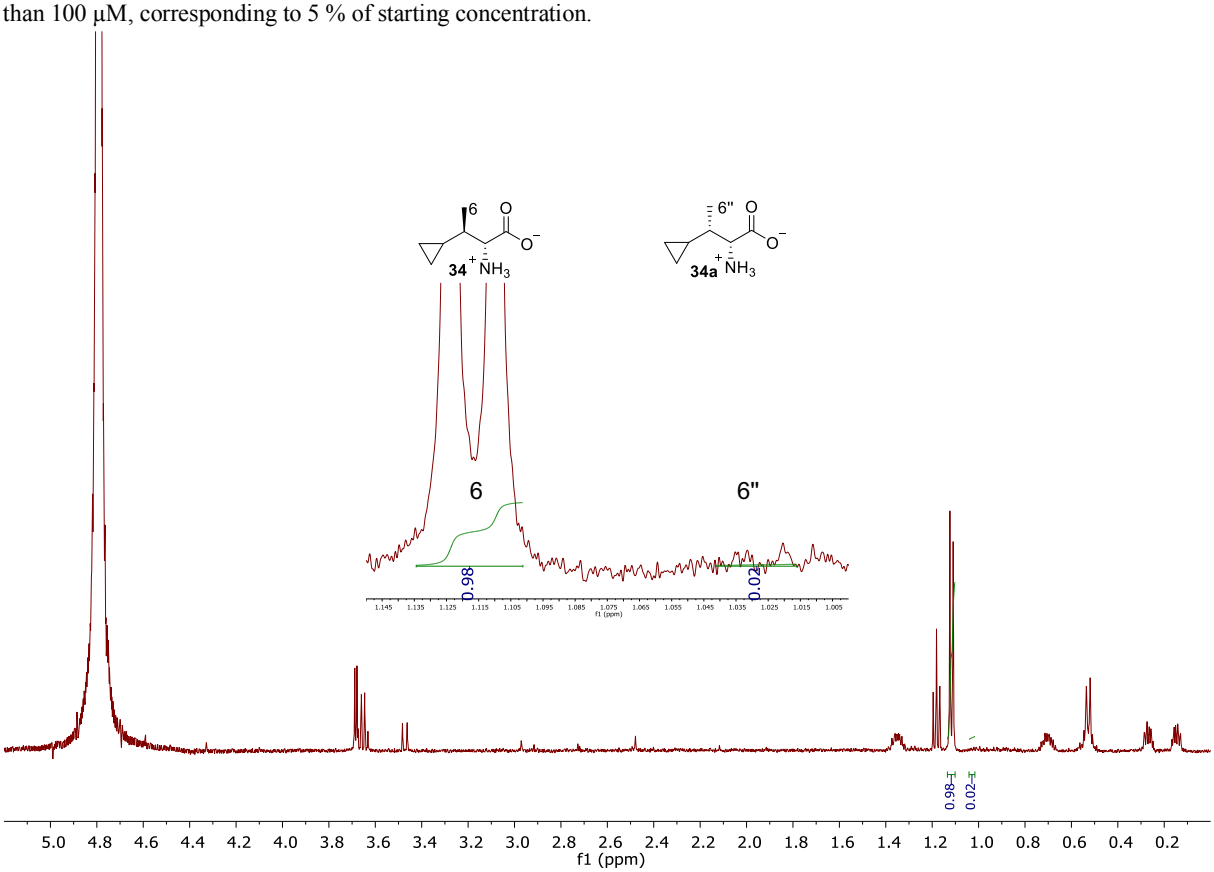
Supplementary Figure 34. Conversion of D-TA-SgvM cascade taking D-methionine (**26**) as substrate Conversion was calculated based on integrals of *S*- and β-methyl groups from both substrate and product. Conversion = 2.44/3 x 100% = 81 %



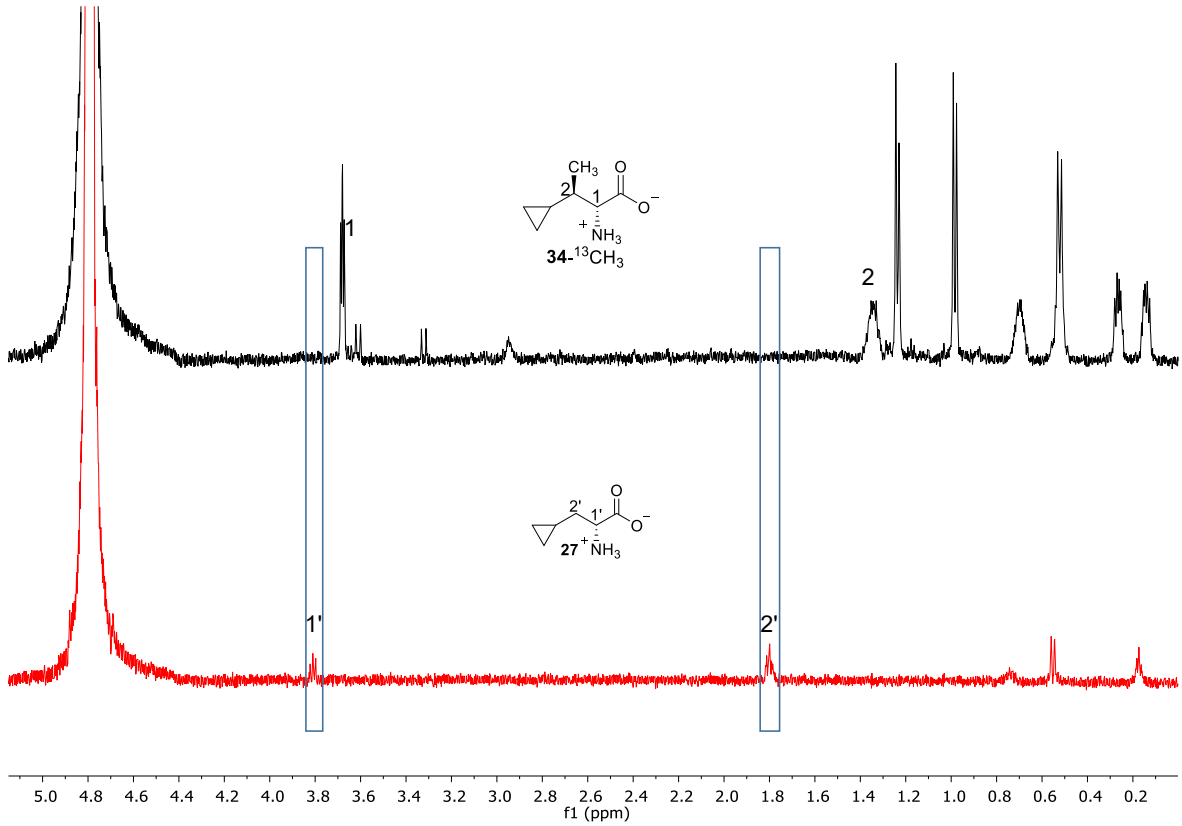
Supplementary Figure 35. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-methionine (**26**) as substrate. Ratio was calculated based on integrals of β-methyl group from both product **33** and putative isomer (95:5).



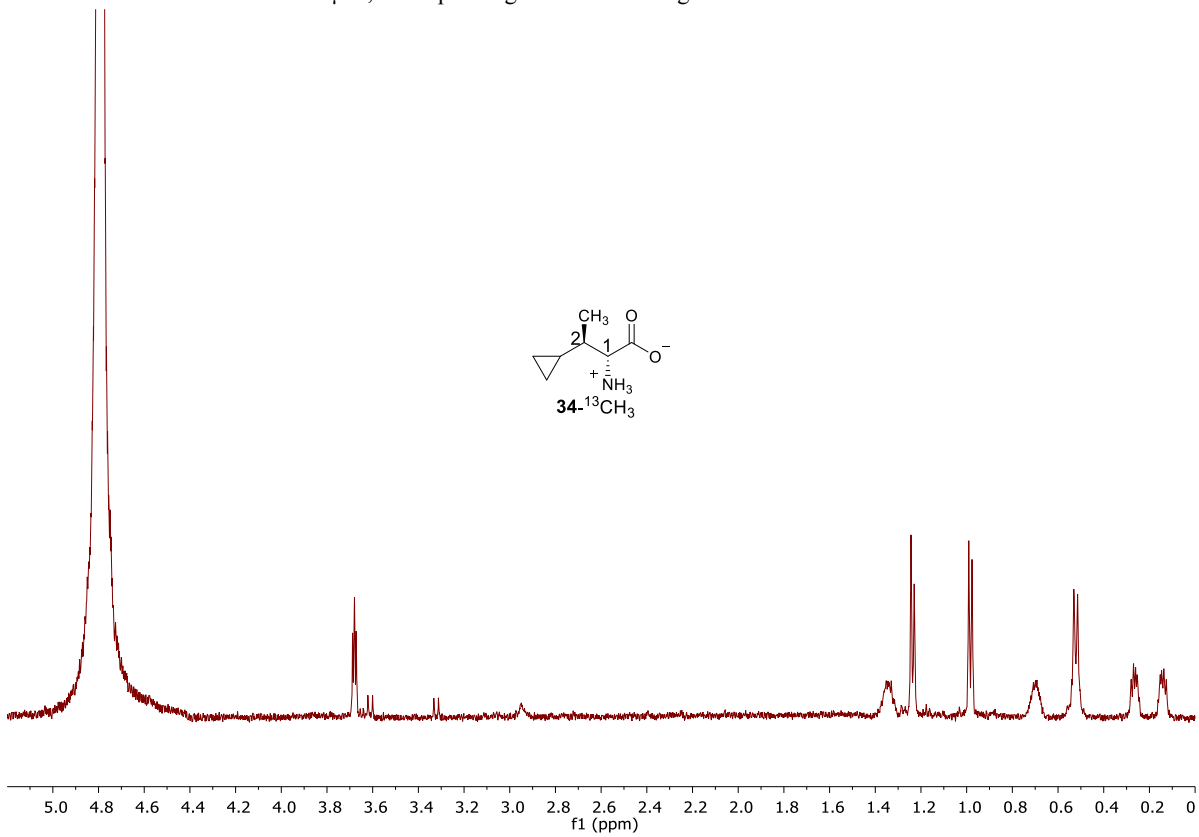
Supplementary Figure 36. Conversion of D-TA-SgvM cascade taking D-cyclopropylalanine (**27**) as substrate. Conversion was determined over 95 %, because no substrate was observed in reaction mixture (**Top**) while resonances was evidently observed in control experiment with 100 µM substrate (**Bottom**). Therefore, residue substrate within reaction mixture is lower than 100 µM, corresponding to 5 % of starting concentration.



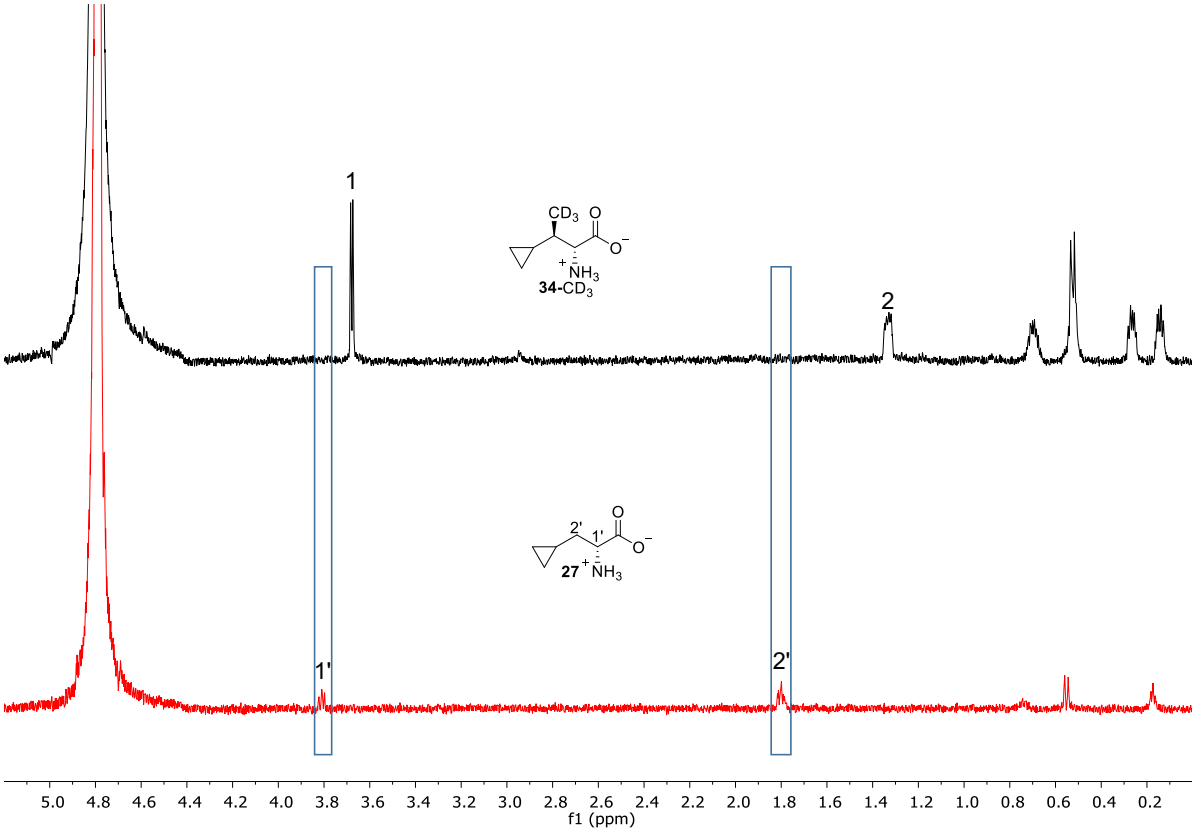
Supplementary Figure 37. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-cyclopropylalanine (**27**) as substrate. Ratio was calculated based on integrals of β-methyl group from both product **34** and putative isomer (98:2).



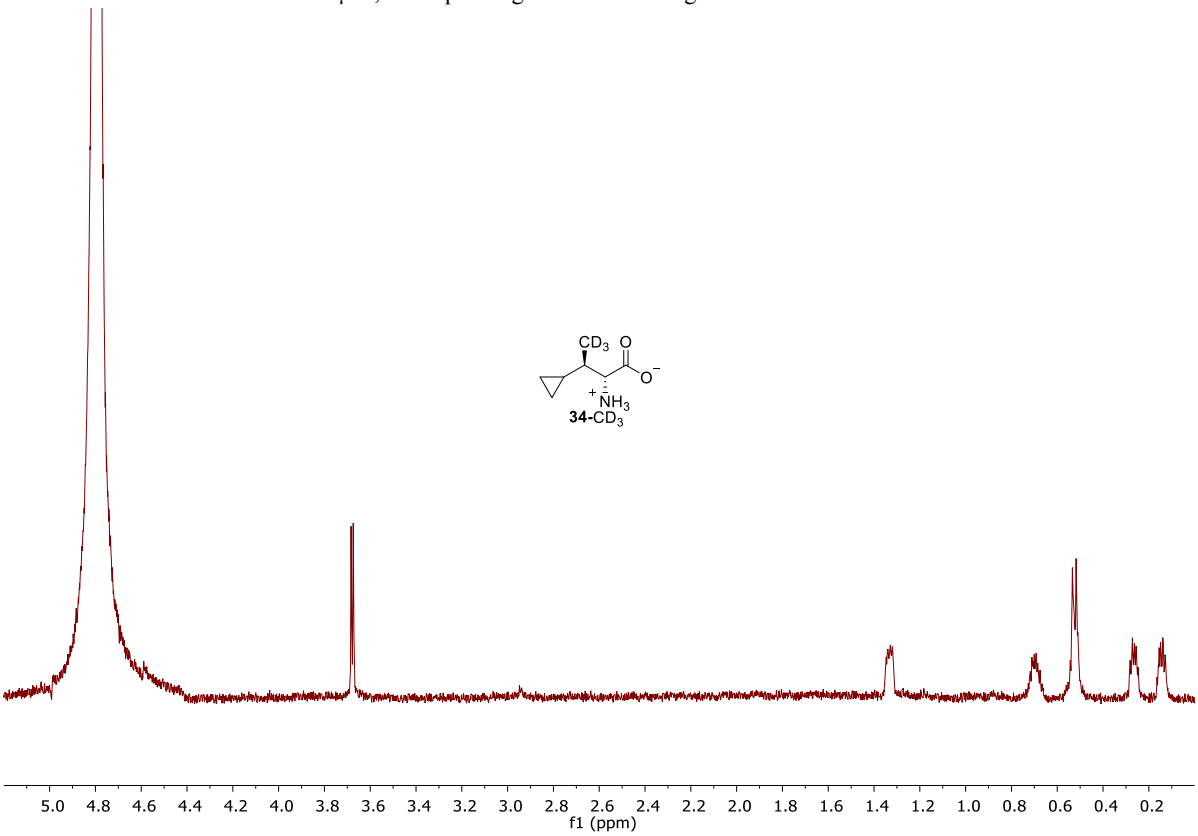
Supplementary Figure 38. Conversion of D-TA-SgvM cascade taking D-cyclopropylalanine (**27**) as substrate and ^{13}C-iodomethane. Conversion was determined over 95 %, because no substrate was observed in reaction mixture (**Top**) while resonances was evidently observed in control experiment with 100 µM substrate (**Bottom**). Therefore, residue substrate within reaction mixture is lower than 100 µM, corresponding to 5 % of starting concentration.



Supplementary Figure 39. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-cyclopropylalanine (**27**) and iodomethane-^{13}C as substrate. No putative isomer signal was observed. The ratio was deduced to be 98 :2, based on standard iodomethane reaction.



Supplementary Figure 40. Conversion of D-TA-SgvM cascade taking D-cyclopropylalanine (**27**) as substrate and iodomethane-d3. Conversion was determined over 95 %, because no substrate was observed in reaction mixture (**Top**) while resonances was evidently observed in control experiment with 100 µM substrate (**Bottom**). Therefore, residue substrate within reaction mixture is lower than 100 µM, corresponding to 5 % of starting concentration.



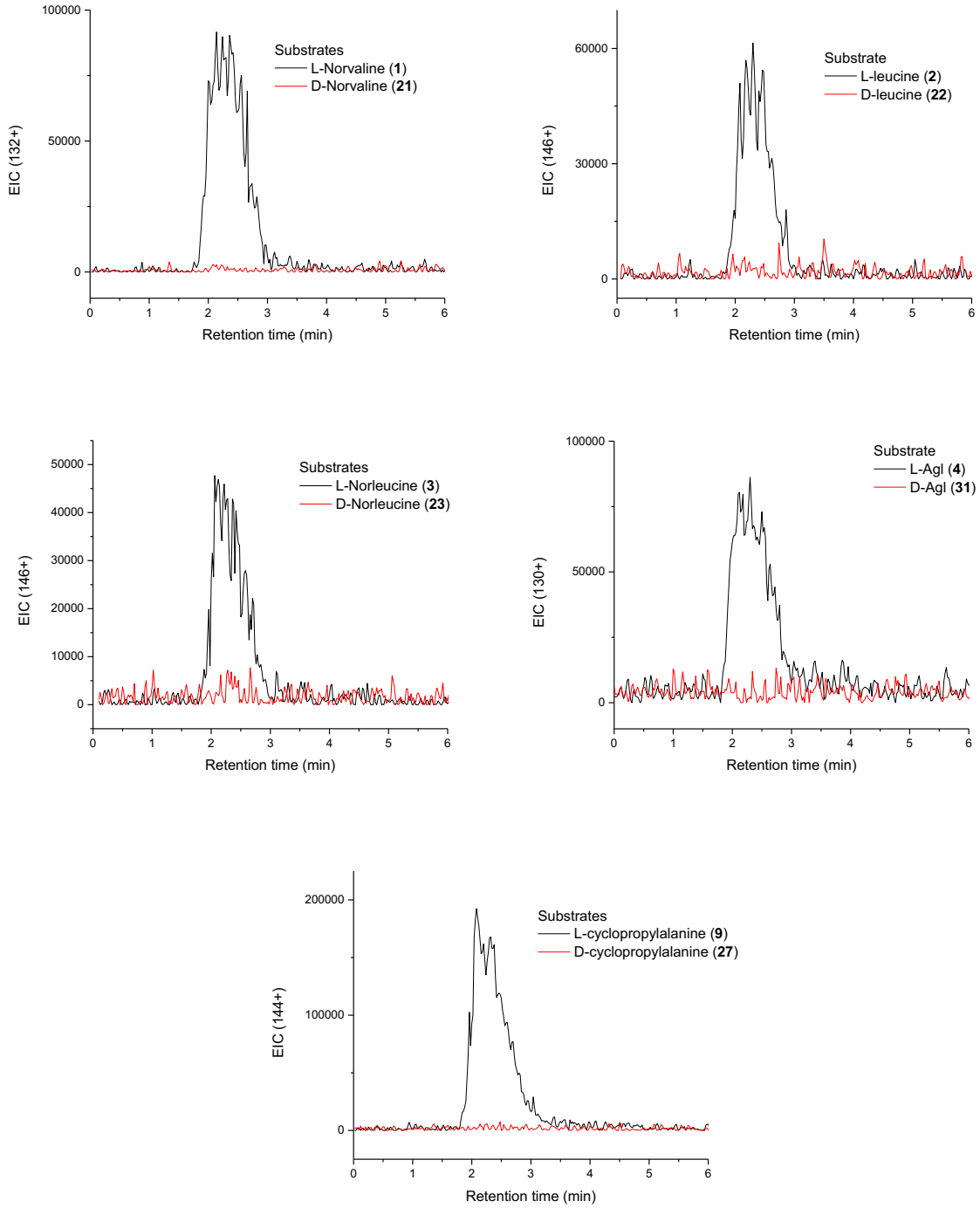
Supplementary Figure 41. 3R:3S ratio of product of D-TA-SgvM cascade reaction using D-cyclopropylalanine (**27**) and iodomethane-d_3 as substrate. No putative isomer signal was observed. The ratio was deduced to be 98:2, based on standard iodomethane reaction.

Origin of stereochemical heterogeneity in enzyme-catalyzed production of β-Me-a-aas

The minor stereochemical heterogeneity in the methylated product could occur because of limited stereoselectivity of the involved enzymes or uncatalyzed racemization. In our previous report on SgvM-catalyzed methylation of alpha-keto acids we showed that uncatalyzed racemization at C3 occurs at an appreciable rate (3×10^{-7} s^{-1}),[1] whereas SgvM-catalyzed methyl transfer occurs with nearly absolute selectivity. The diastereomeric ratio of the final product (β-Me-α-aa) is therefore a function of this rate and the steady state concentration of the methylated keto acid intermediate. The concentration of this intermediate is dependent on the concentration of PLP (bound to TA and unbound). Because the D-TA was less active than IlvE (L-TA) we used higher PLP and TA concentrations for the D-α-amino acid (described above). Therefore, it is not surprising that methylation of D-α-amino acid produced product with slightly smaller diastereomeric ratios.

We also considered the possibility that limited stereoselectivity of the TAs could lead to the observed minor diastereomers. To test this idea, we fed the IlvE/SgvM/HMT cascade with D-α-amino acid (norvaline, leucine, norleucine, allylglycine) and examined the products by mass spectrometry. In contrast to reactions with L-α-amino acids we could not detect any product in the D-α-amino acid containing reactions (Figure S42). The complementary experiment with D-TA and L-α-amino acids produced equivalent results (Figure S43). These observations provide no evidence for stereochemical heterogeneity caused by L- or D-TAs. Hence, we conclude that the minor isomer in the produced β-Me-α-aas contains S configuration at C3 (Table 1).

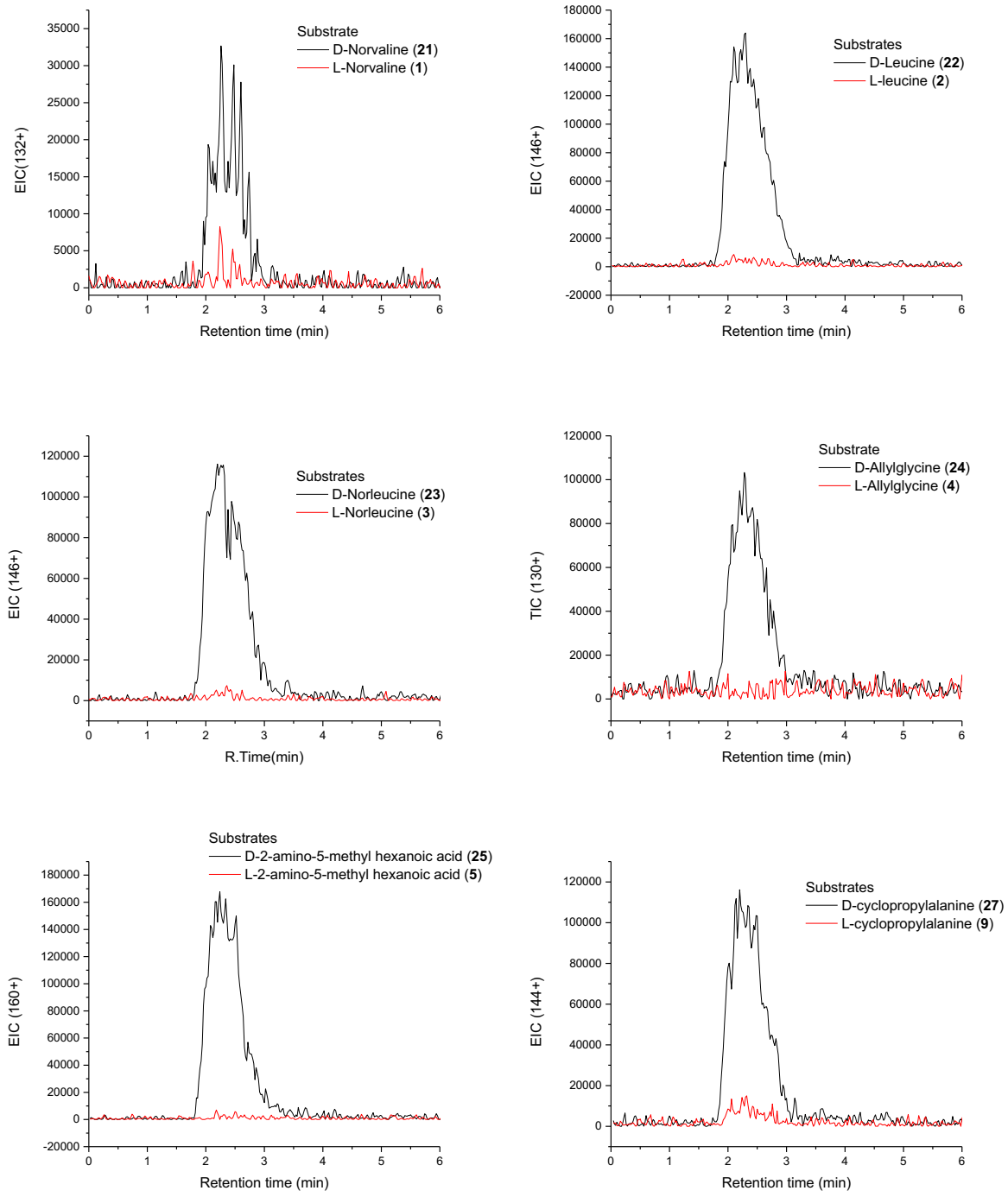
The IlvE/SgvM/HMT cascade does not methylate D-α-amino acid. 0.1 ml reactions containing 2 mM of L- or D-α-amino acids, 4 mM methyl iodide, 40 μM SAH, 40 μM PLP, 40 μM IlvE, 40 μM SgvM, 20 μM HMT and 100 mM sodium phosphate buffer (pH = 8.0) were incubated at room temperature. After 24 hours, 20 μL of the reaction mixture was dilute with 980 μL of methanol. After filtration with 0.45 μm syringe filter, the sample were analyzed by mass spectrometry. Extracted-ion chromatograms of these measurements show that L-α-amino acids were methylated, but D-α-amino acids were not.



Supplementary Figure 42. Extracted ion chromatogram (EIC) of IlvE cascades accepting L- or D-α-amino acids. Substrates were indicated in each figure. It is shown that D-α-amino acids were not methylated by IlvE .

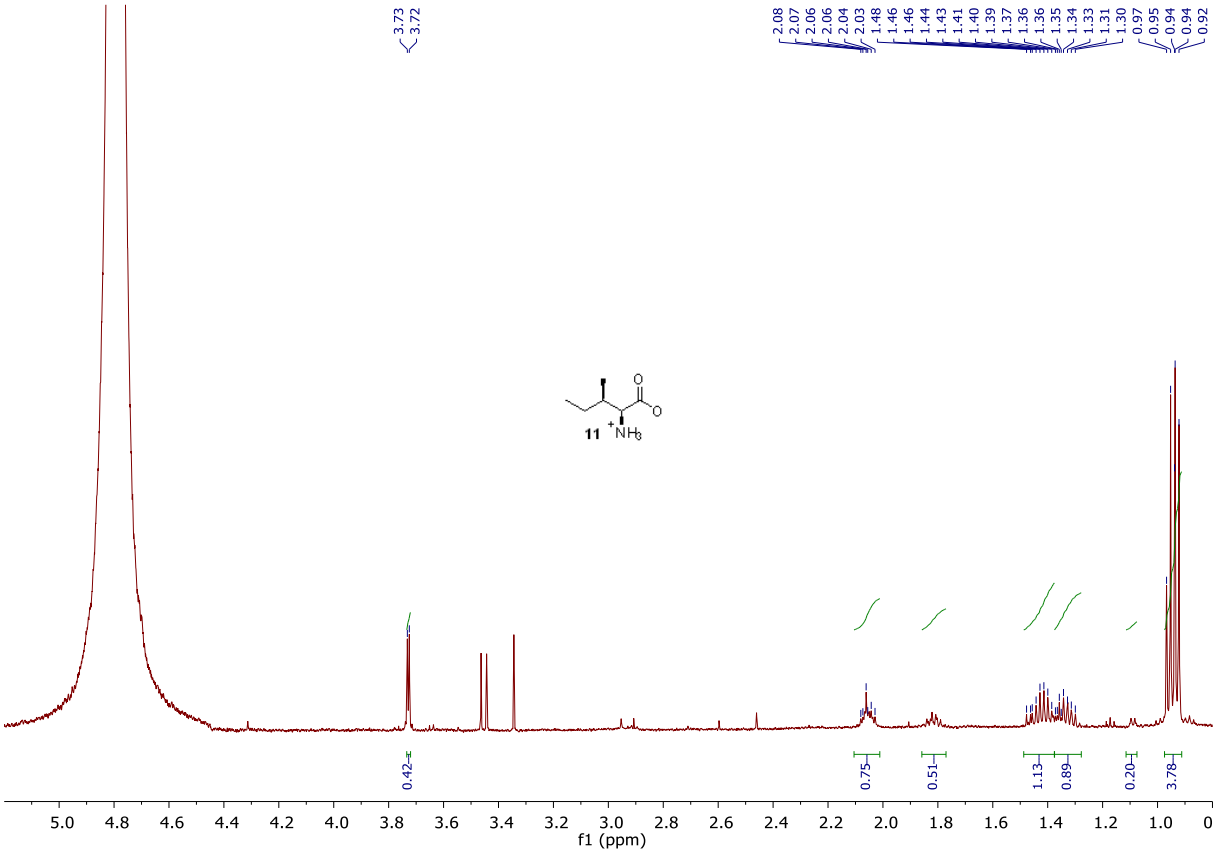
The D-TA/SgvM/HMT cascade does not methylate L-α-amino acid.

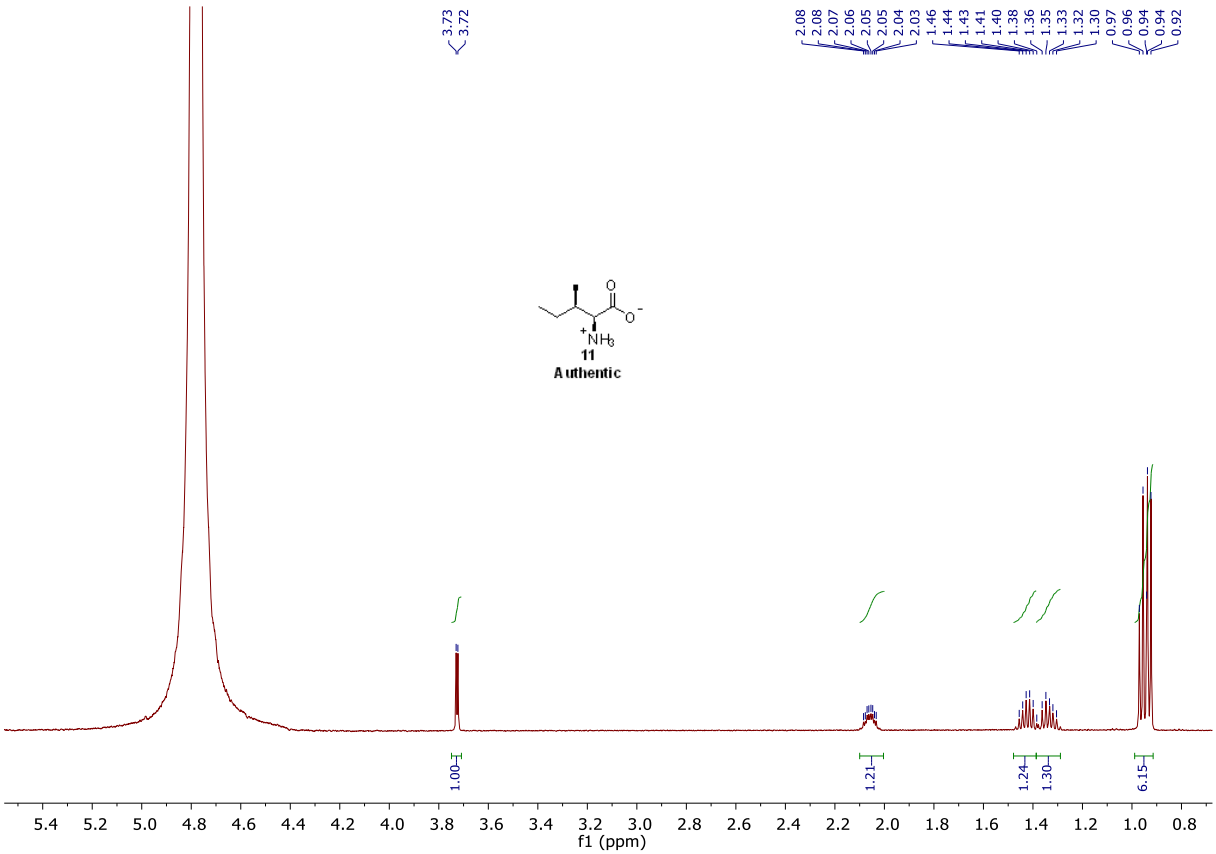
0.1 ml reactions containing 2 mM of L- or D-α-amino acids, 4 mM methyl iodide, 40 μM SAH, 80 μM PLP, 80 μM D-TA, 40 μM SgvM, 20 μM HMT and 100 mM sodium phosphate buffer (pH = 8.0) were incubated at room temperature. After 24 hours, 20 μL of the reaction mixture was dilute with 980 μL of methanol. After filtration with 0.45 μm syringe filter, the sample were analyzed by mass spectrometry. Extracted-ion chromatograms of these measurements show that D-α-amino acids were methylated, but L-α-amino acids were not.

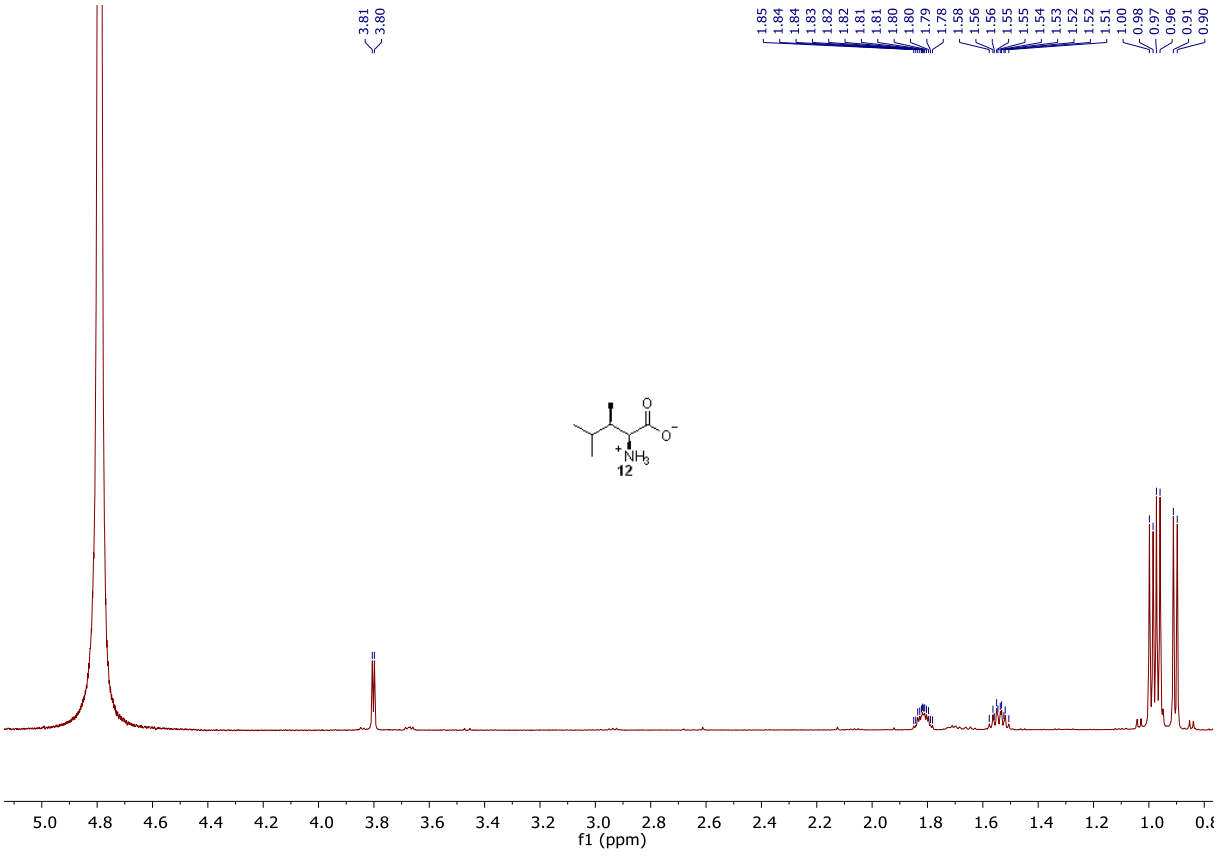


Supplementary Figure 43. Extracted ion chromatograms (EIC) of D-TA cascades accepting L- or D-α-amino acids. Substrates were indicated in each figure. It is shown that L-α-amino acids were not methylated by D-TA.

1D and 2D NMR Spectra of products



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1.84
1.84
1.83
1.82
1.82
1.81
1.81
1.80
1.80
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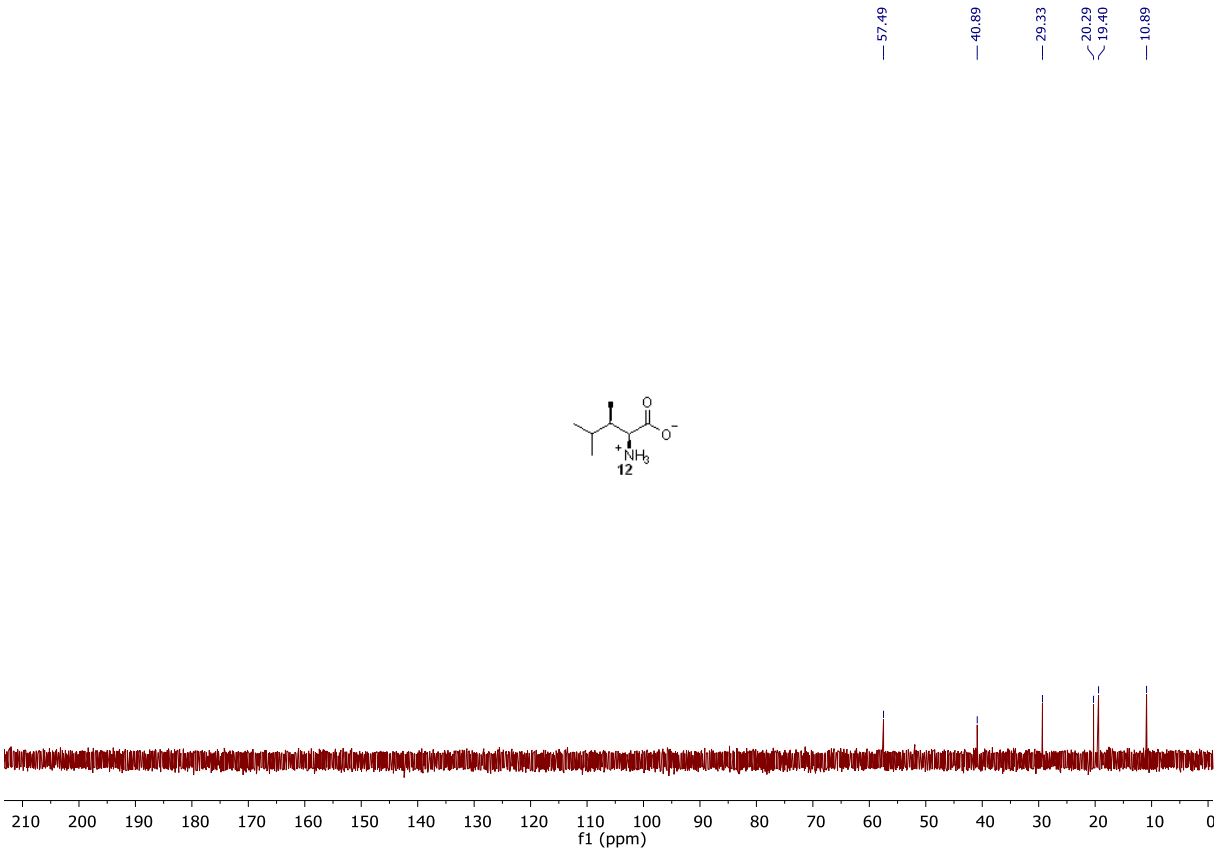
1.78
1.58
1.56
1.56
1.55
1.55
1.54
1.53
1.52
1.52
1.51
1.00
0.98
0.97
0.96
0.91
0.90
O
O⁻
⁺NH₃
12
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
f1 (ppm)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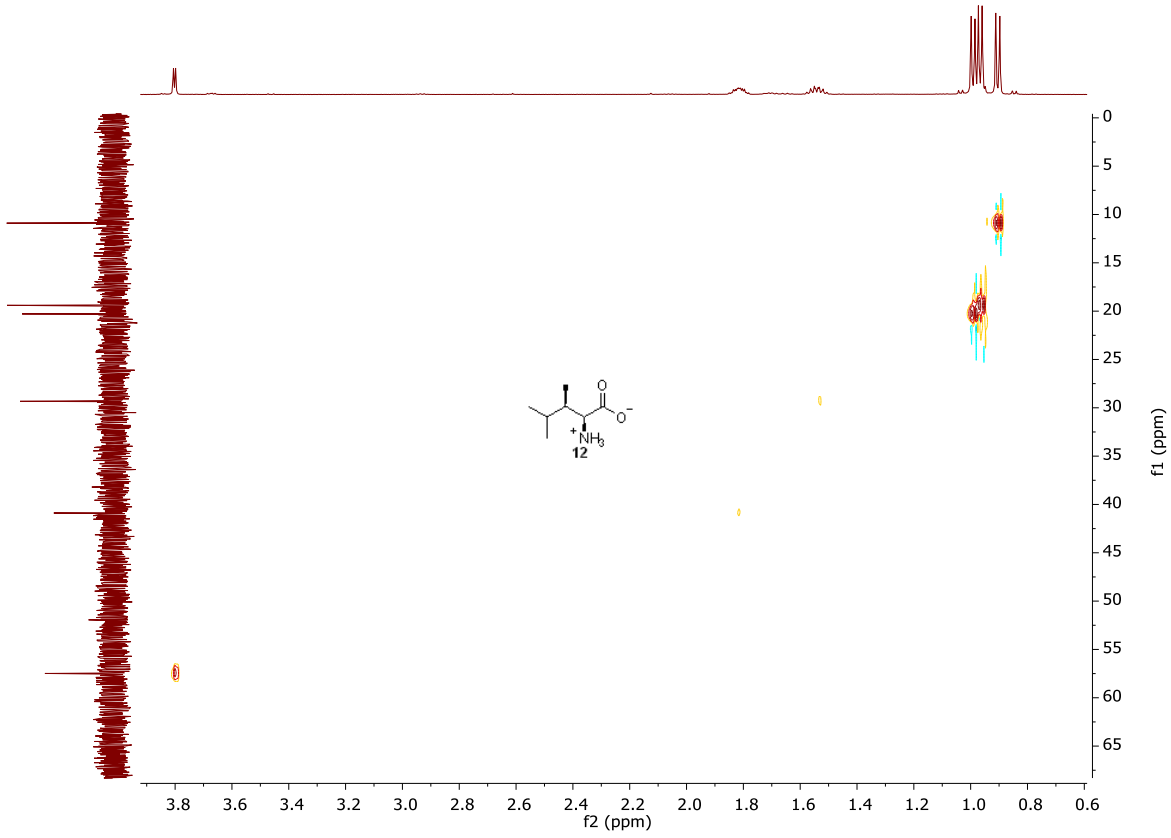
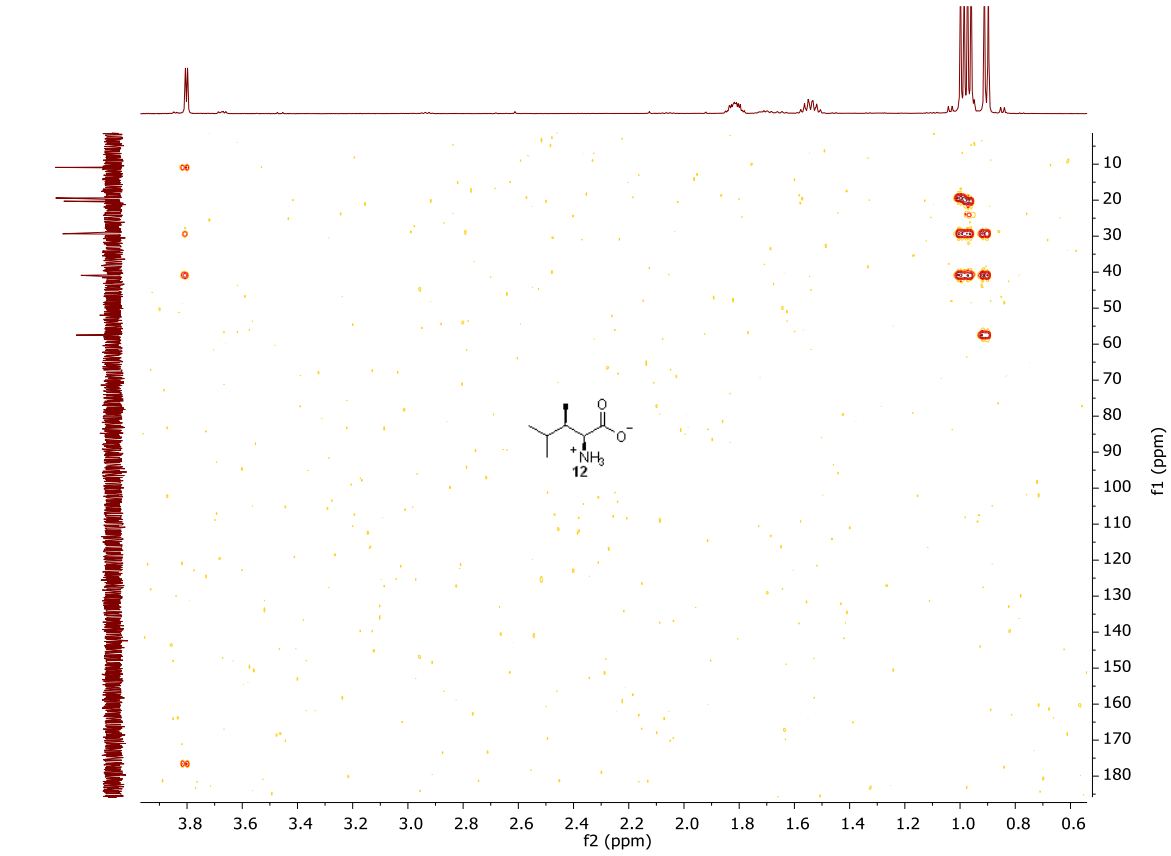
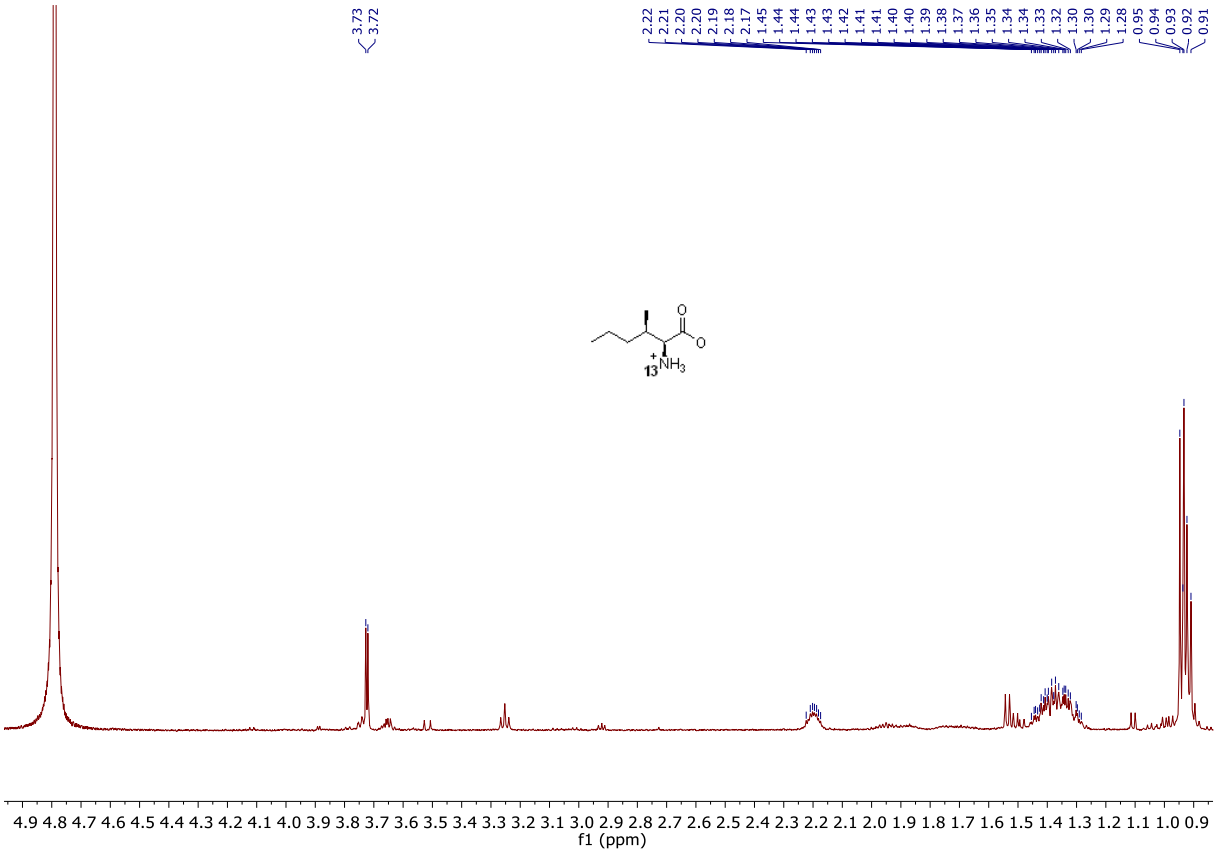
40.89
29.33
20.29
19.40
10.89
O
O⁻
⁺NH₃
12
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


12
f2 (ppm)
f1 (ppm)


12
f2 (ppm)
f1 (ppm)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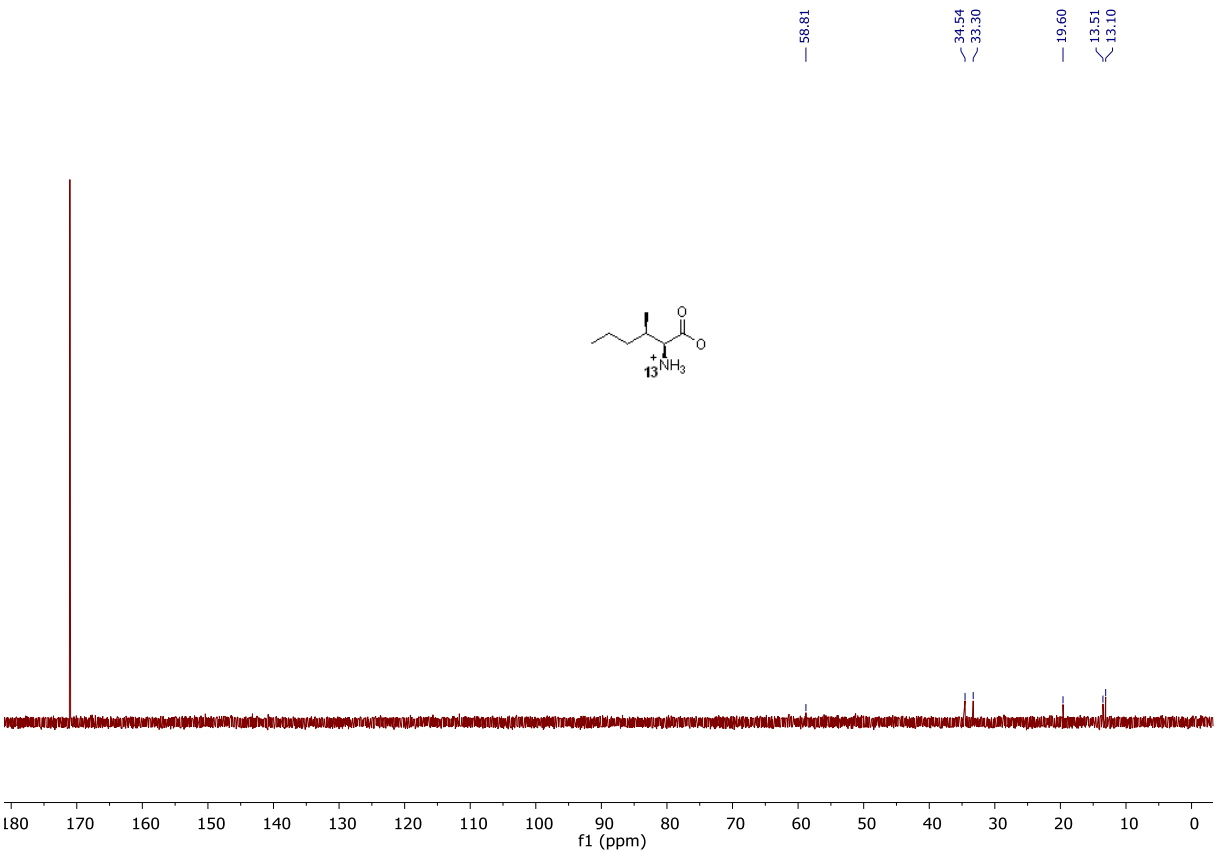
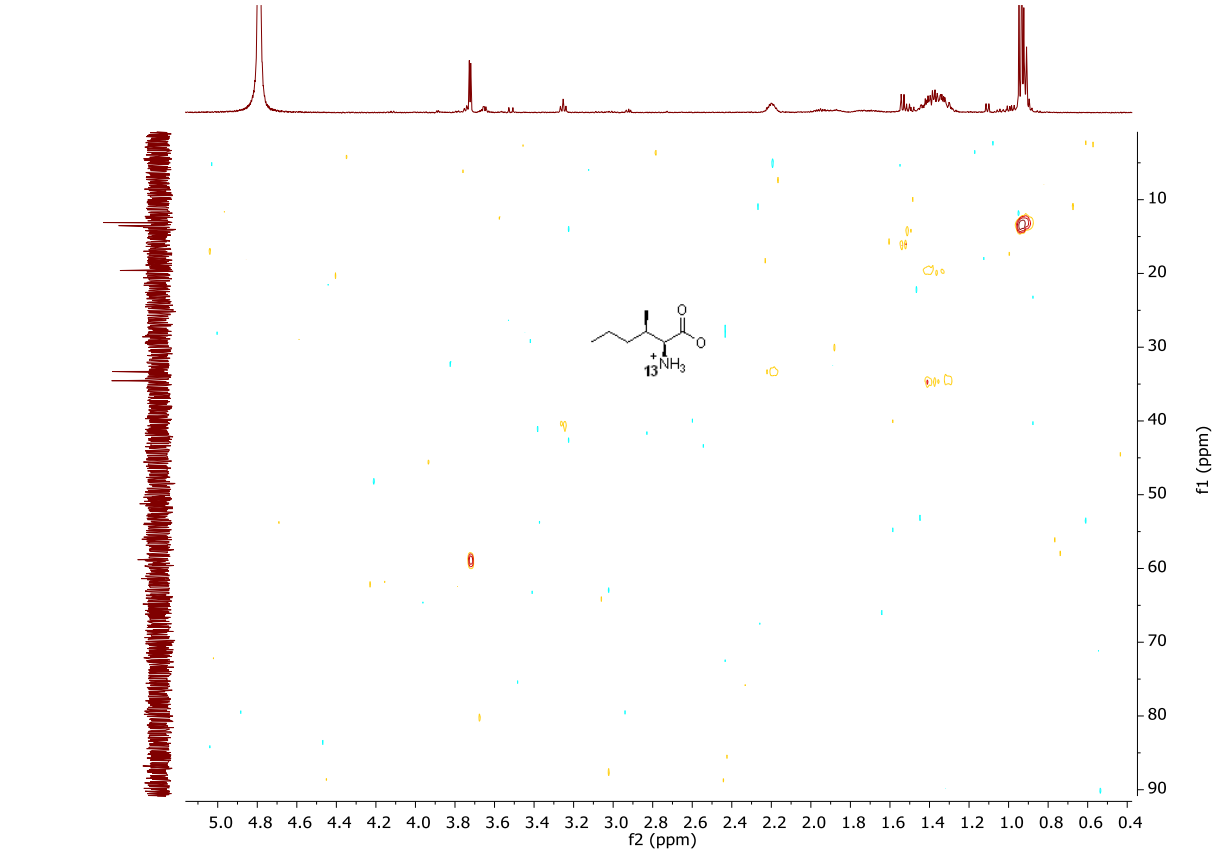
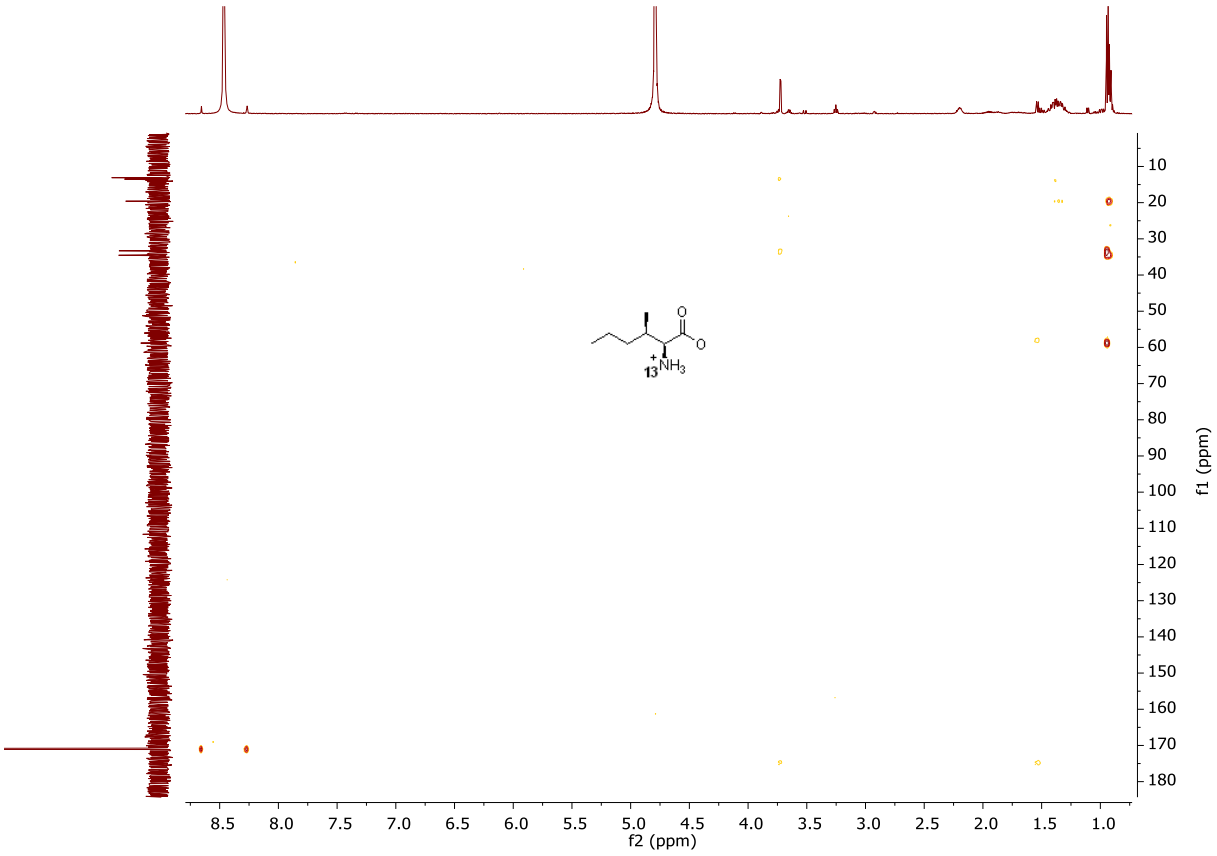
2.20
2.20
2.19
2.18
2.17
1.45
1.44
1.44
1.43
1.43
1.42
1.41
1.41
1.40
1.40
1.39
1.38
1.37
1.36
1.35
1.34
1.34
1.33
1.32
1.30
1.30
1.29
1.28
0.95
0.94
0.93
0.92
0.91
4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2 2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9
f1 (ppm)


58.81
34.54
33.30
19.60
13.51
13.10
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4
f2 (ppm)
10 20 30 40 50 60 70 80 90
f1 (ppm)
13
NH3


8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f2 (ppm)
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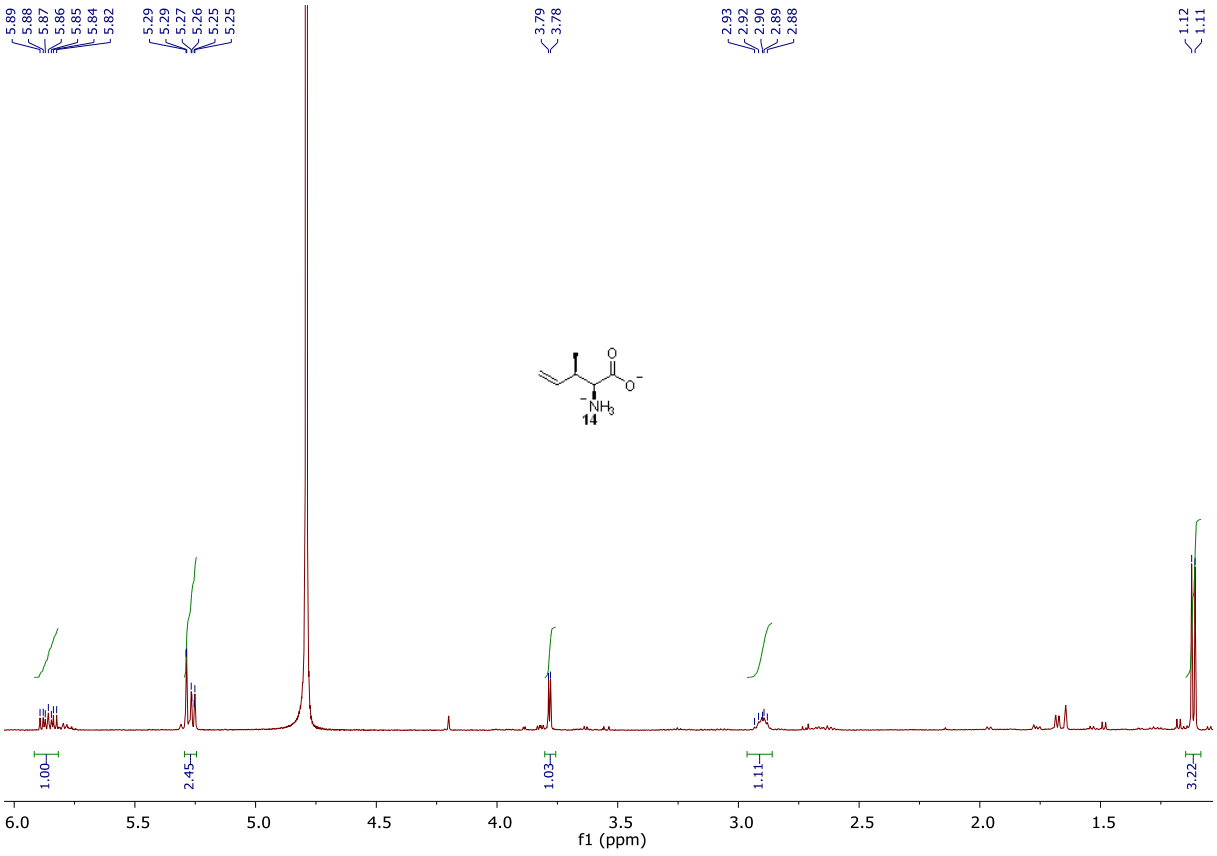
f1 (ppm)
13
NH3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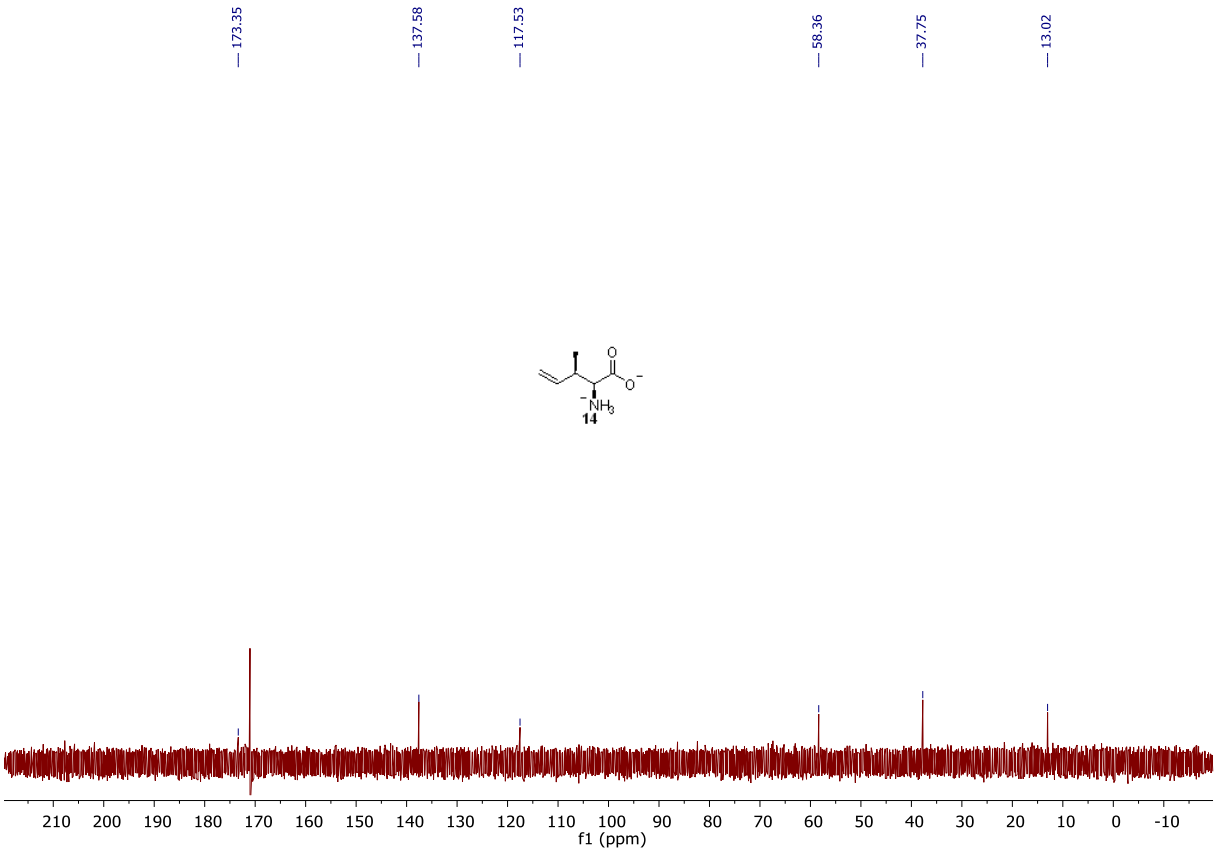
5.86
5.85
5.84
5.82
5.29
5.29
5.27
5.26
5.25
5.25
3.79
3.78
2.93
2.92
2.90
2.89
2.88
1.12
1.11
14
1.00
2.45
1.03
1.11
3.22
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f1 (ppm)


173.35
137.58
117.53
58.36
37.75
13.02
14
210
200
190
180
170
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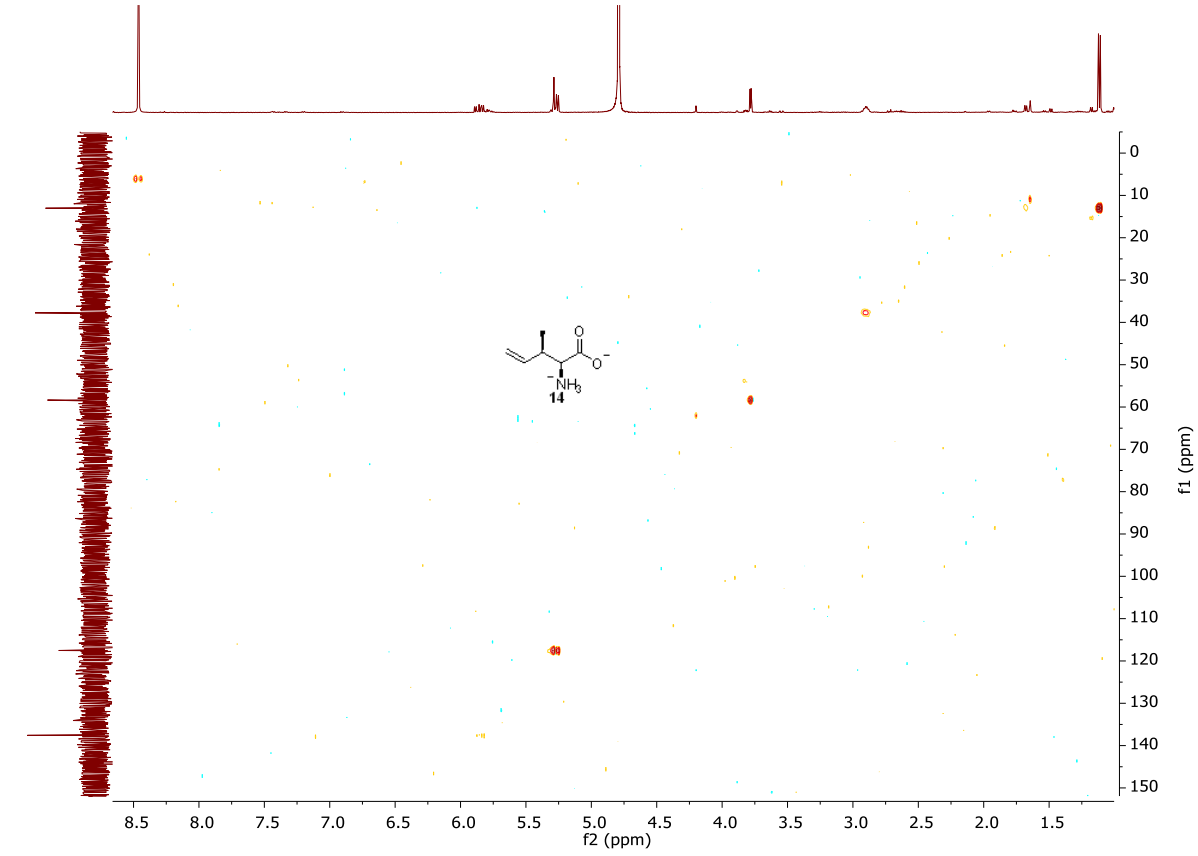
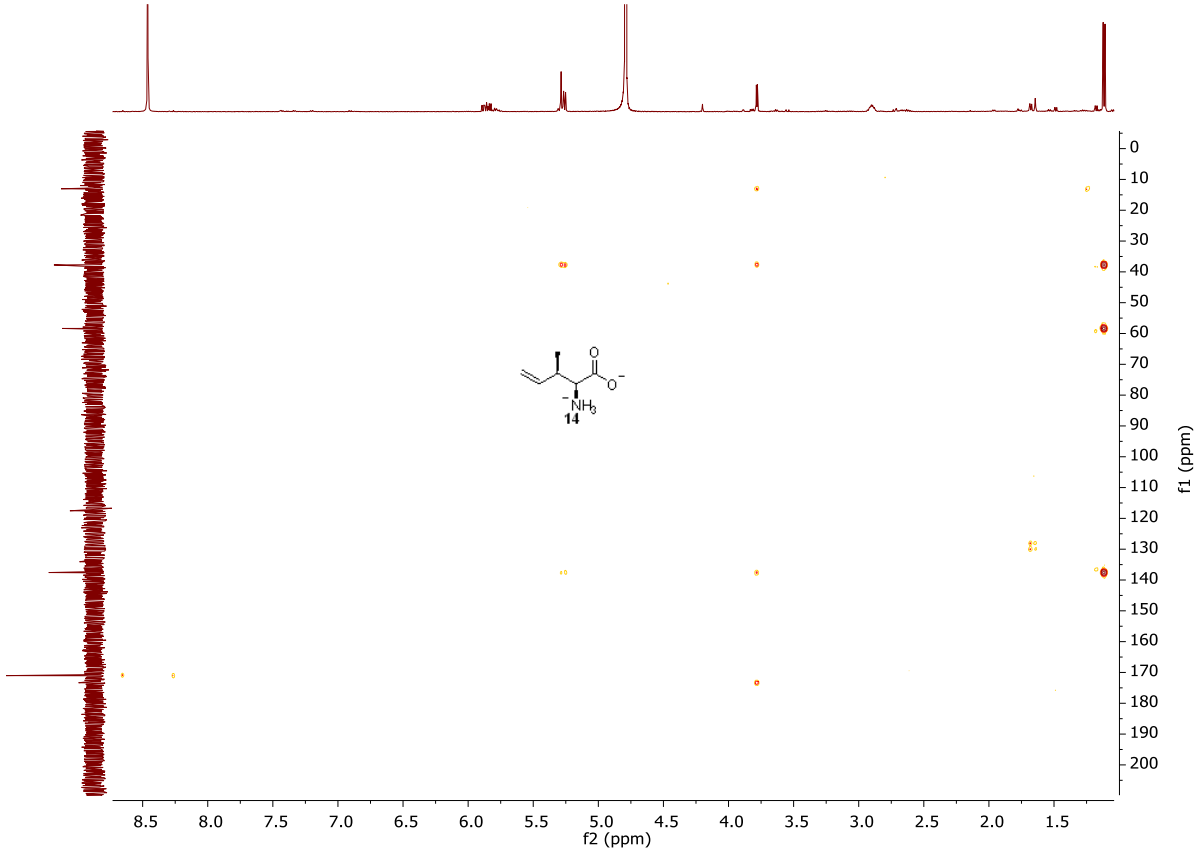
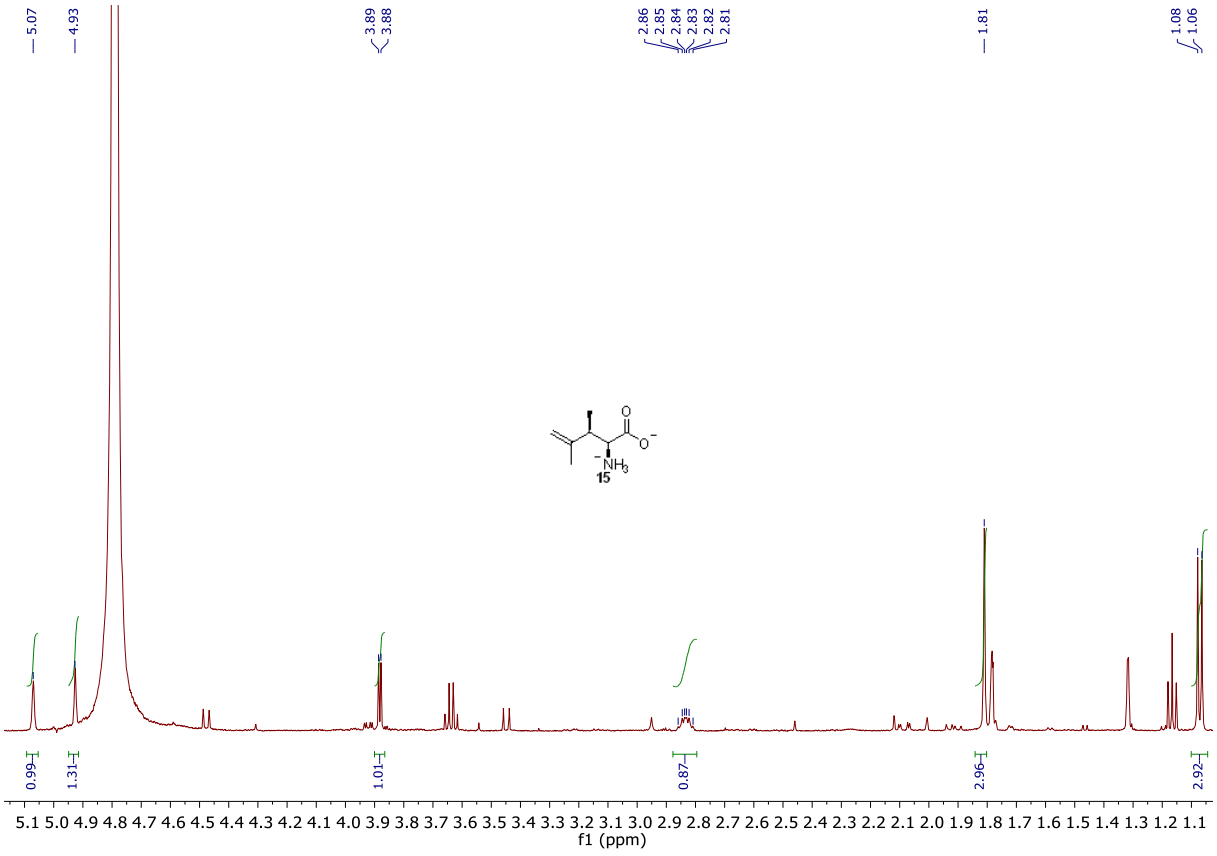
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


f1 (ppm)
f2 (ppm)
14


f1 (ppm)
f2 (ppm)
14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3.88
2.86
2.85
2.84
2.83
2.82
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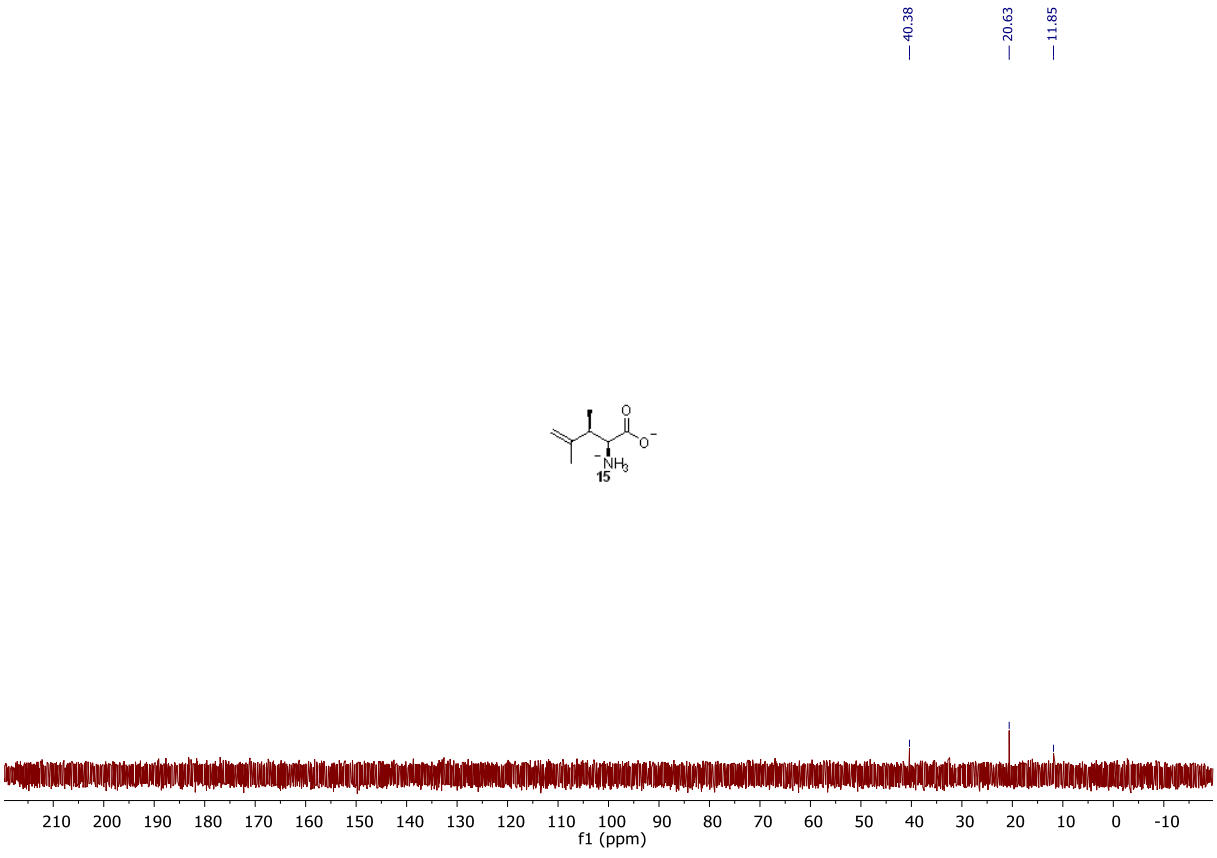
1.81
1.08
1.06
15
0.99
1.31
1.01
0.87
2.96
2.92
f1 (ppm)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15
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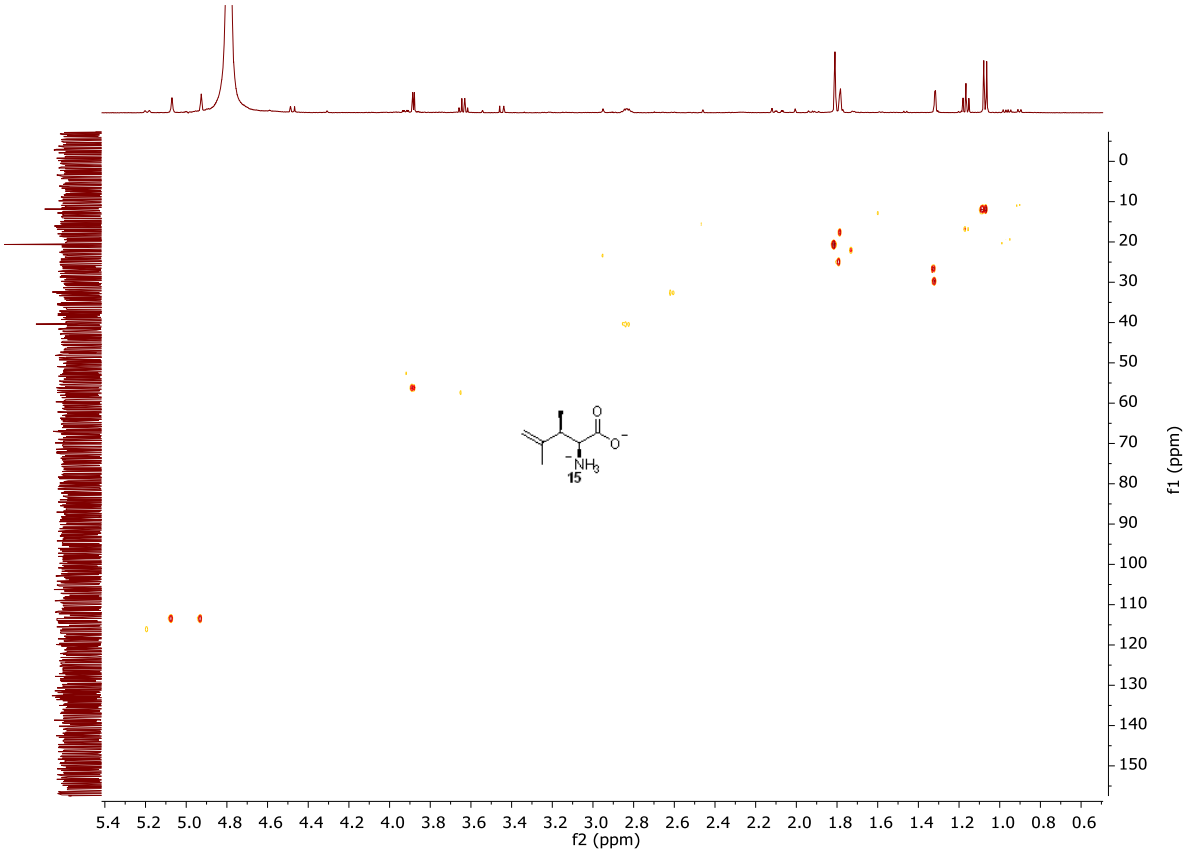
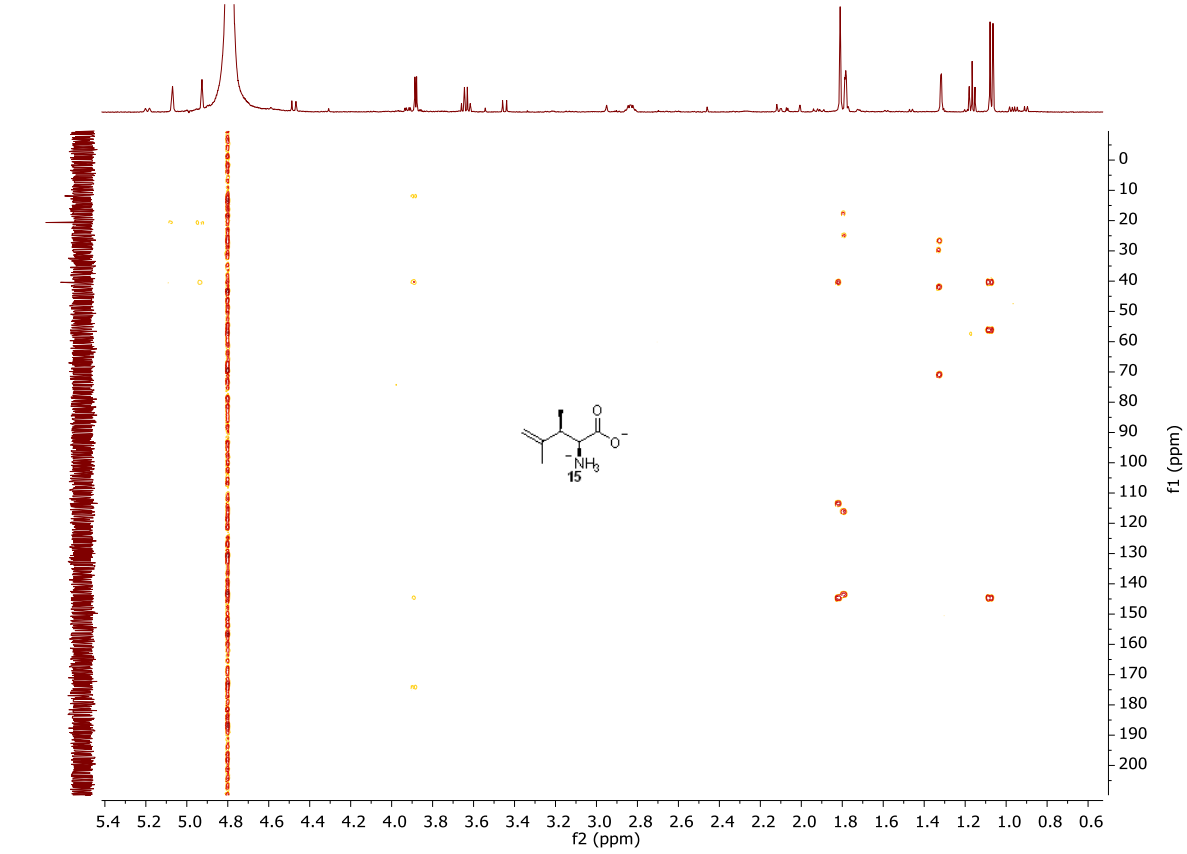

f1 (ppm)
f2 (ppm)
15


f1 (ppm)
f2 (ppm)
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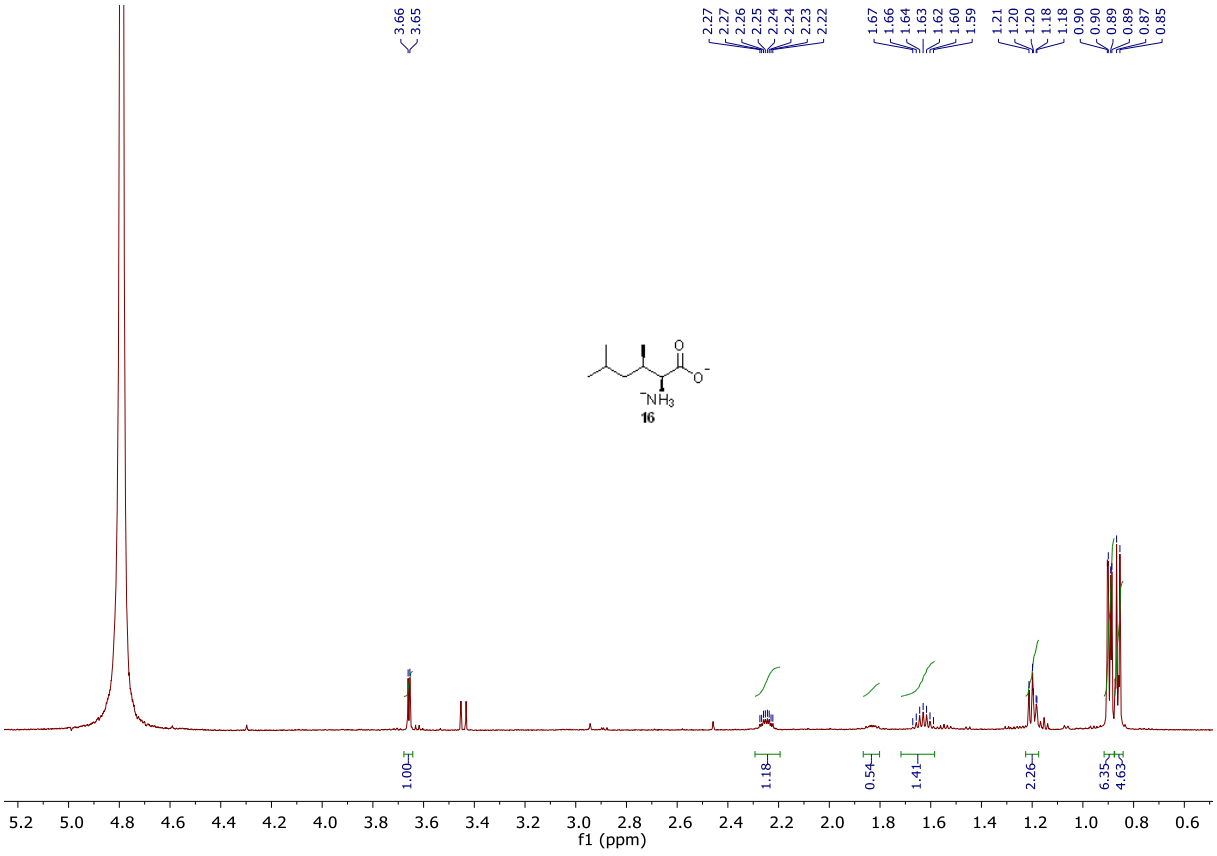
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2.22
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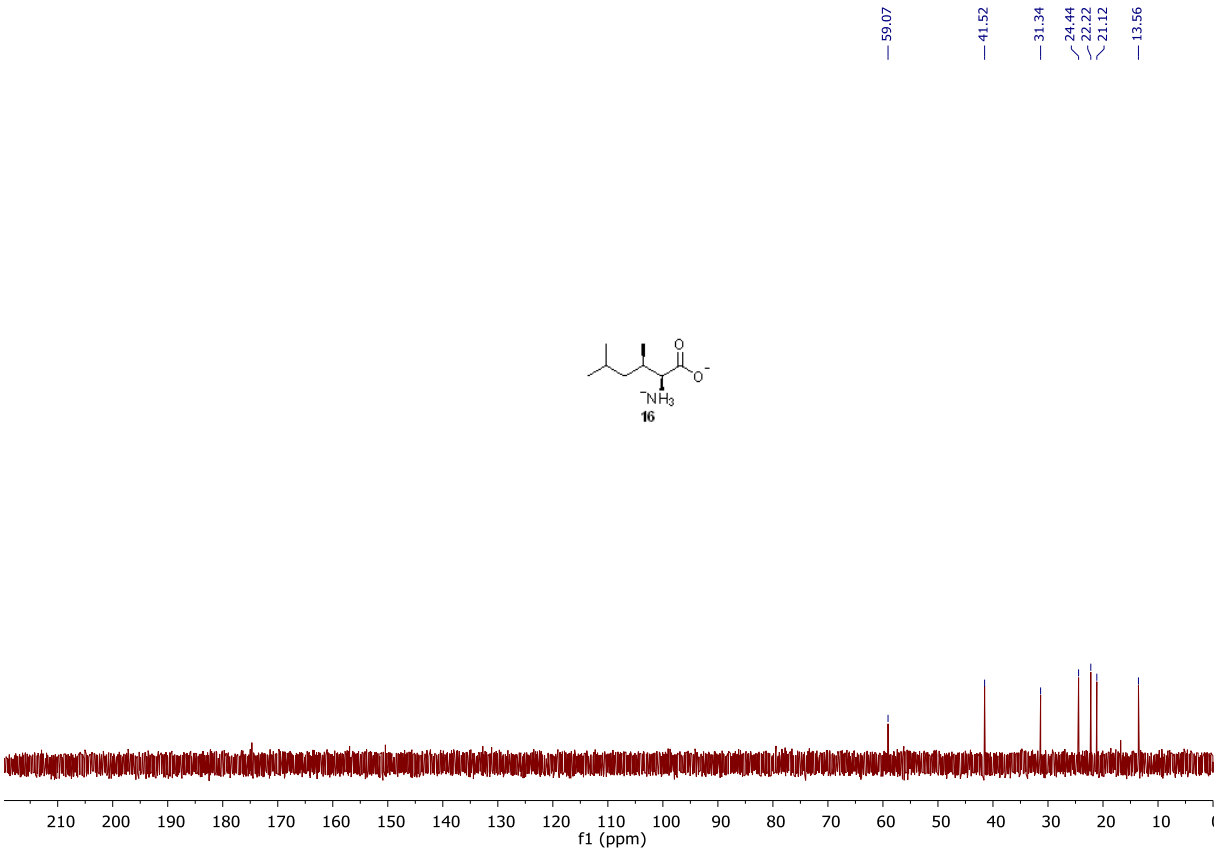
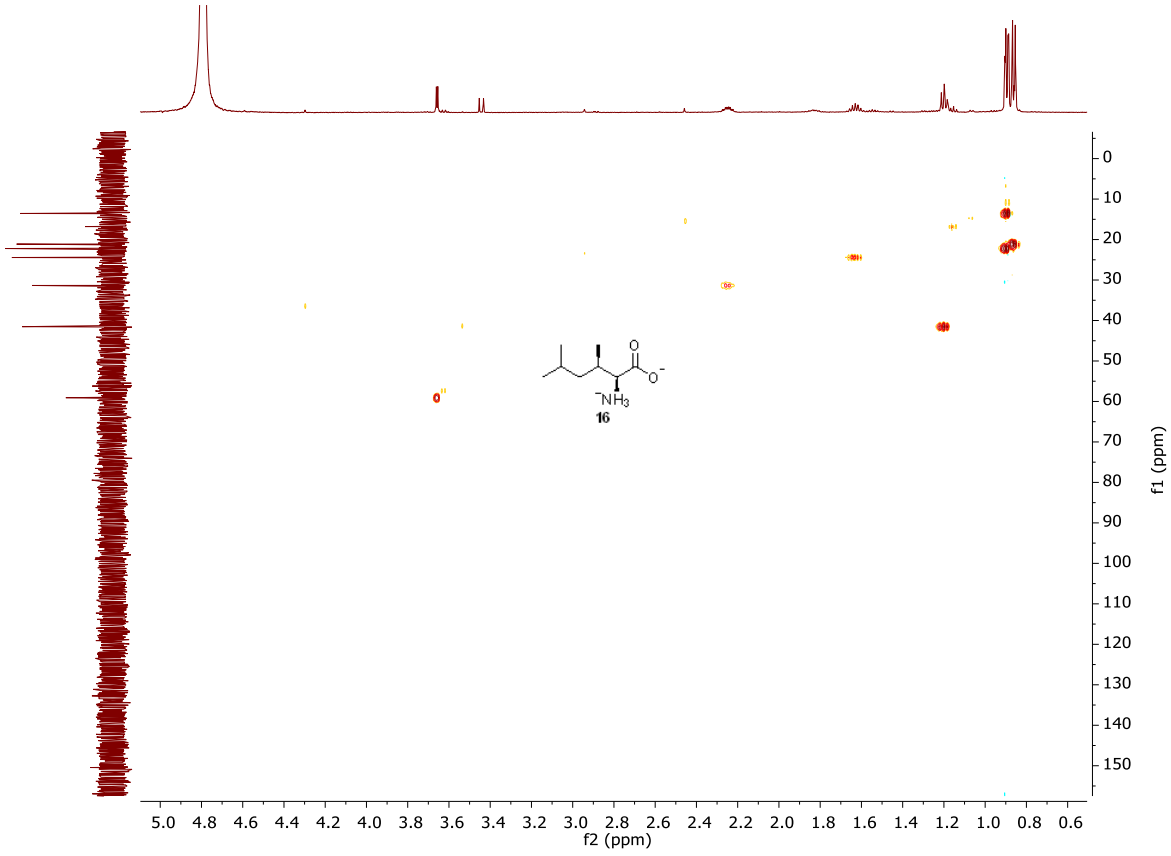
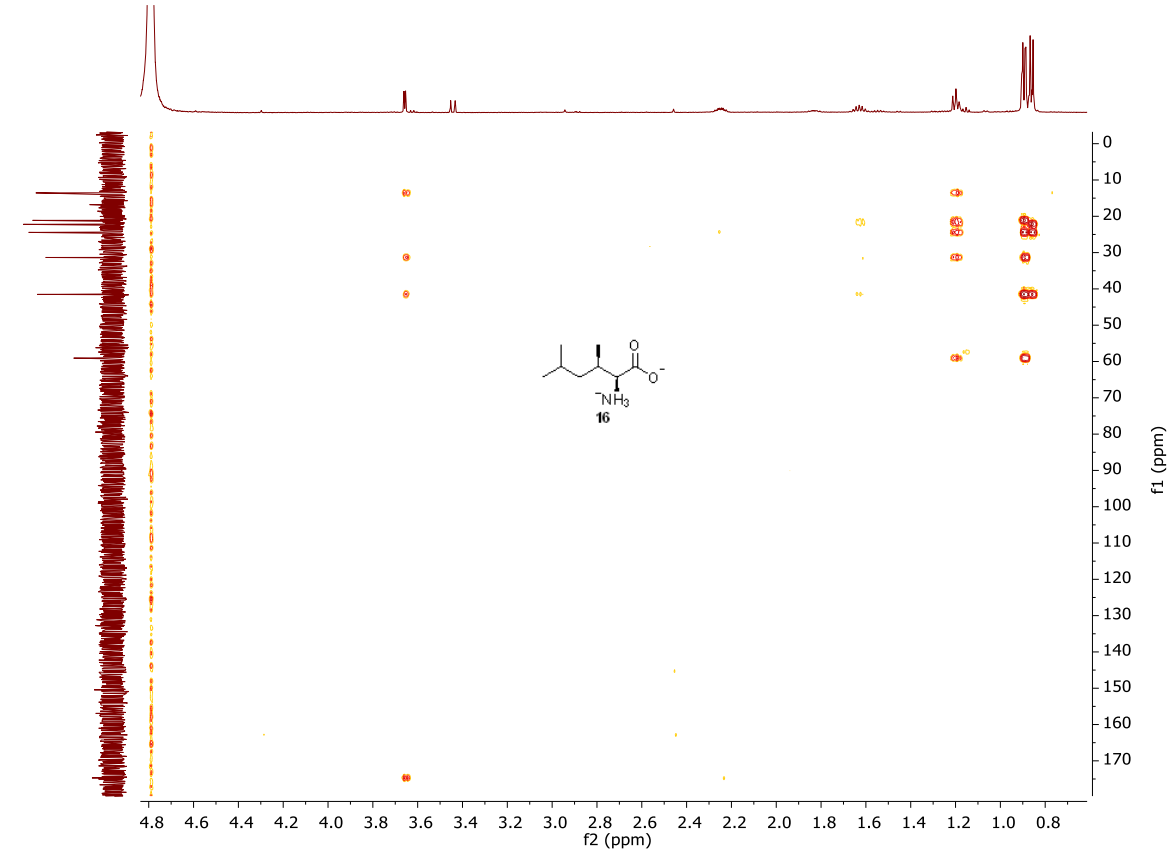
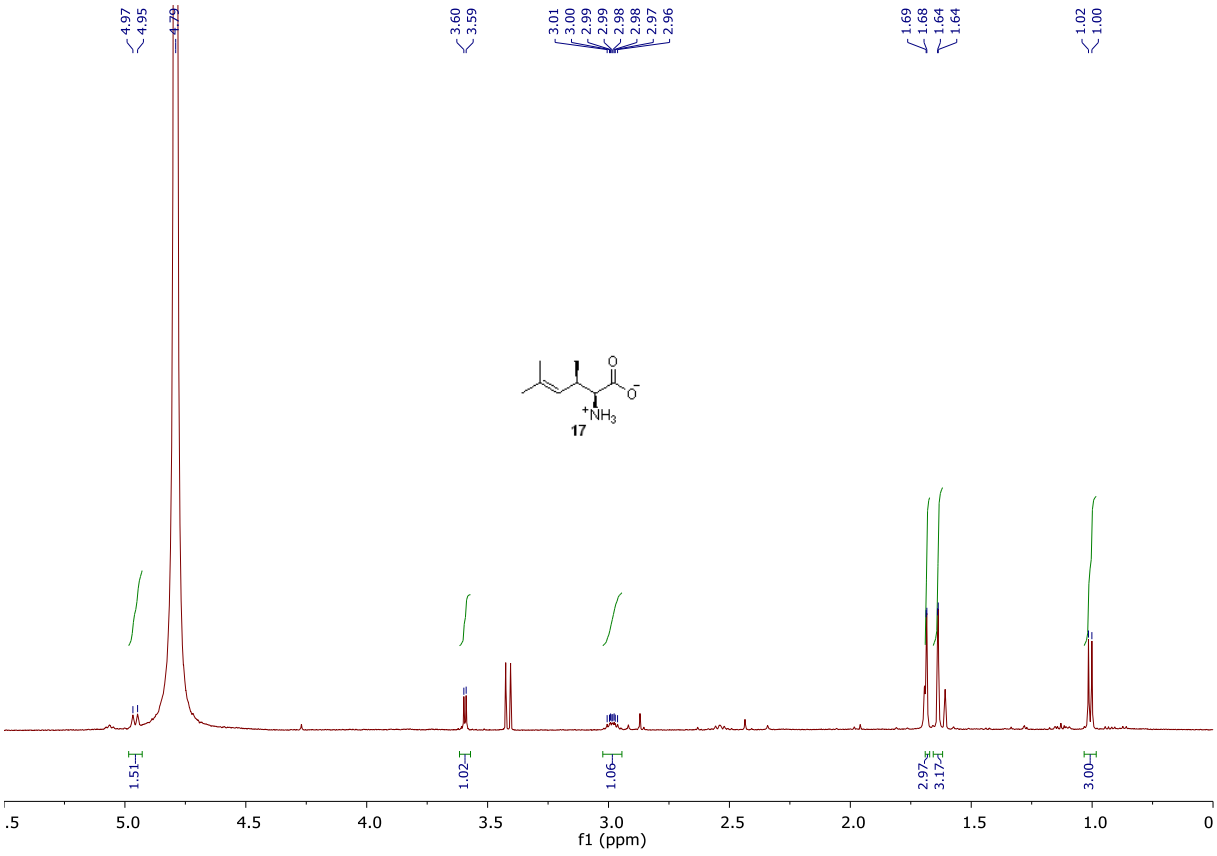
1.66
1.64
1.63
1.62
1.60
1.59
1.21
1.20
1.20
1.18
1.18
0.90
0.90
0.89
0.89
0.87
0.85
O
O-
+NH3
16
1.00
1.18
0.54
1.41
2.26
6.35
4.63
5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6
f1 (ppm)


59.07
41.52
31.34
24.44
22.22
21.12
13.56
O
O-
+NH3
16
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


f1 (ppm)
f2 (ppm)
16


f1 (ppm)
f2 (ppm)
16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2.98
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2.97
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1.69
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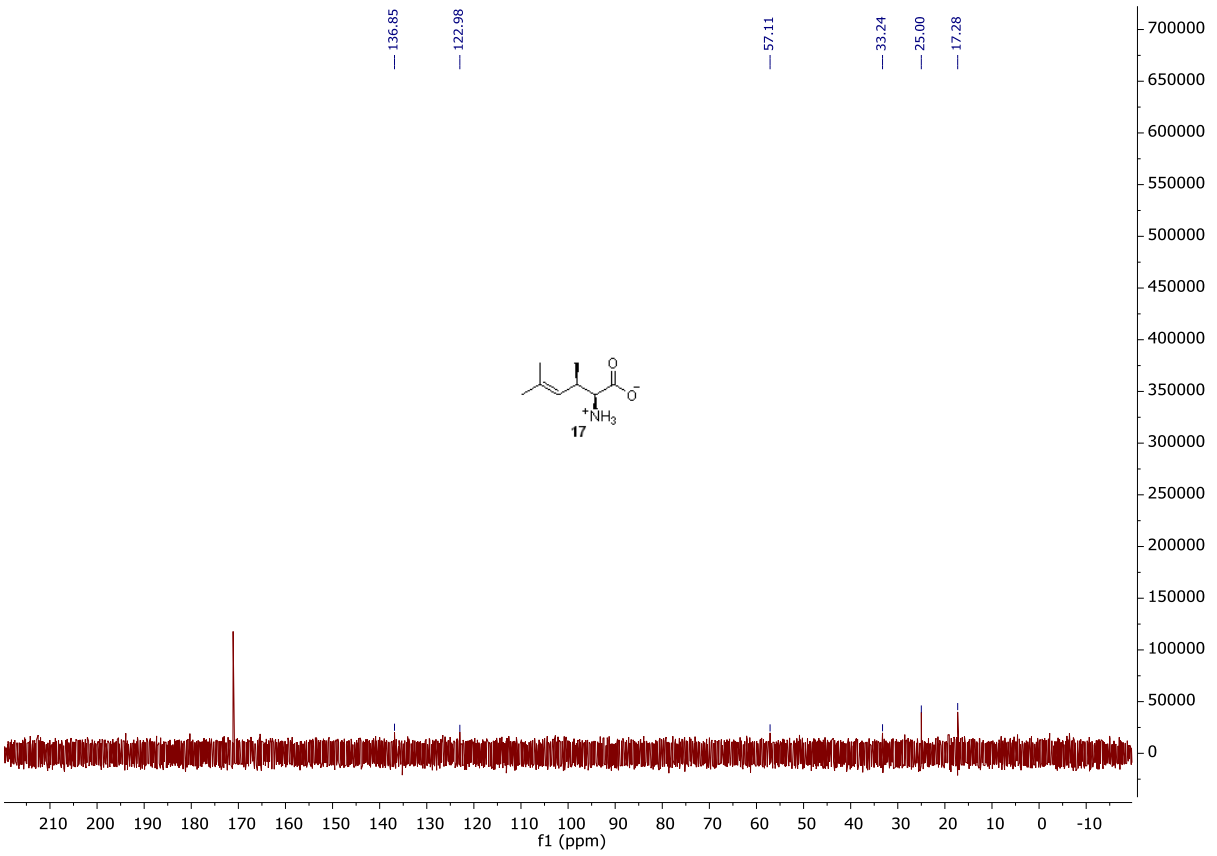
1.06
2.97
3.17
3.00
f1 (ppm)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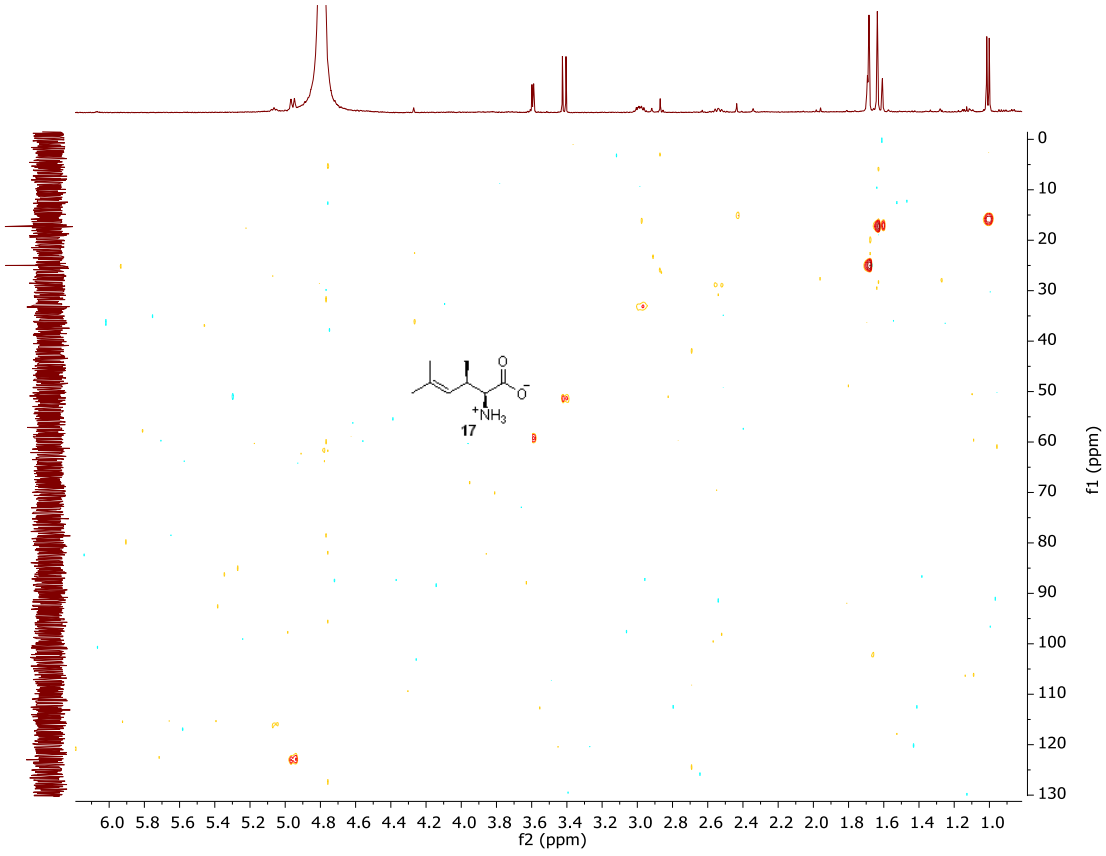
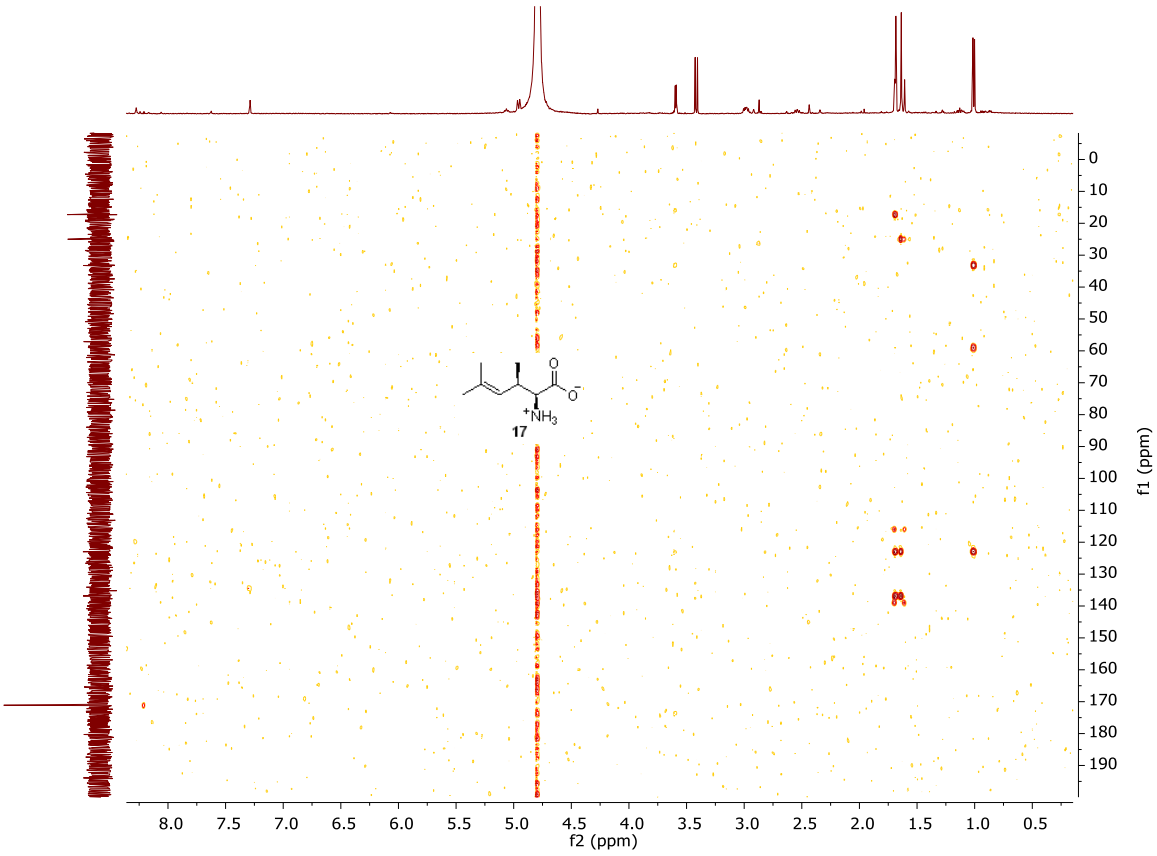
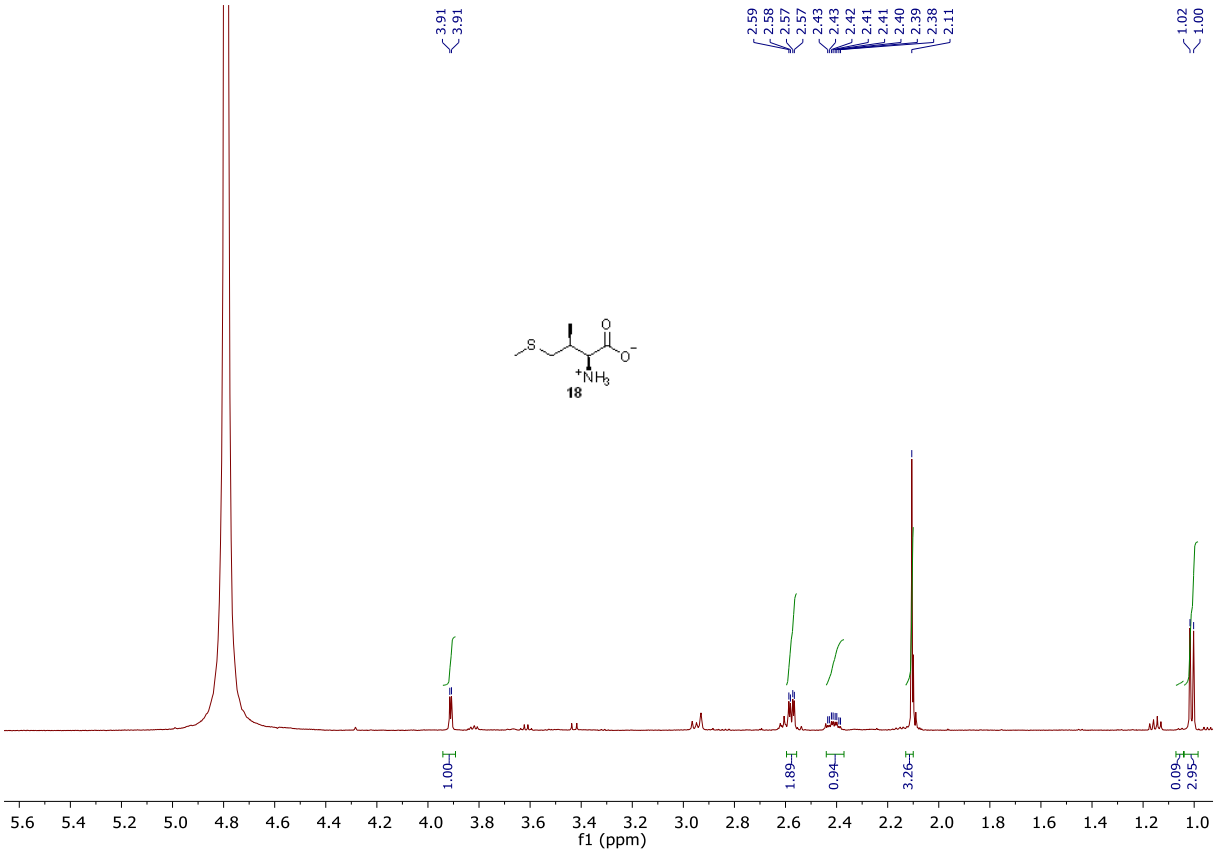
57.11
33.24
25.00
17.28
17
f1 (ppm)


17
f2 (ppm)
f1 (ppm)


17
f2 (ppm)
f1 (ppm)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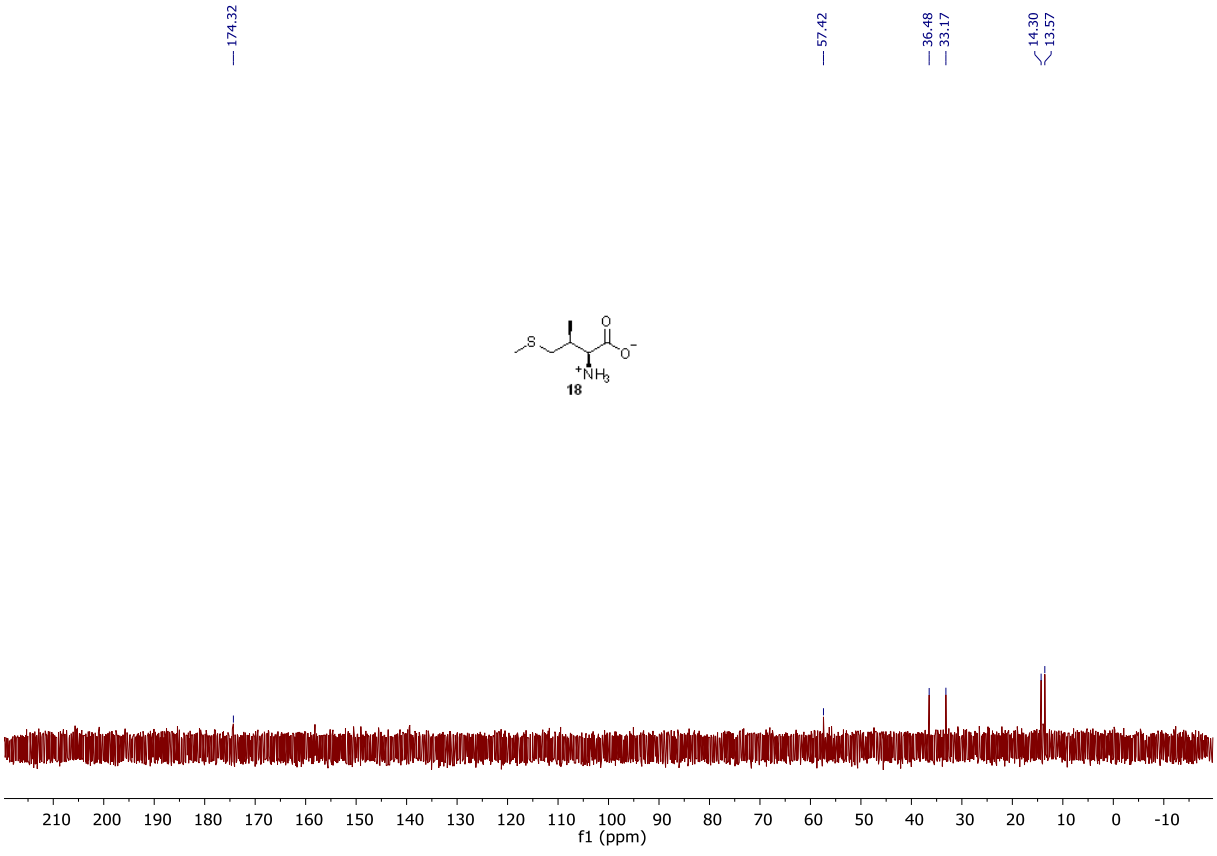
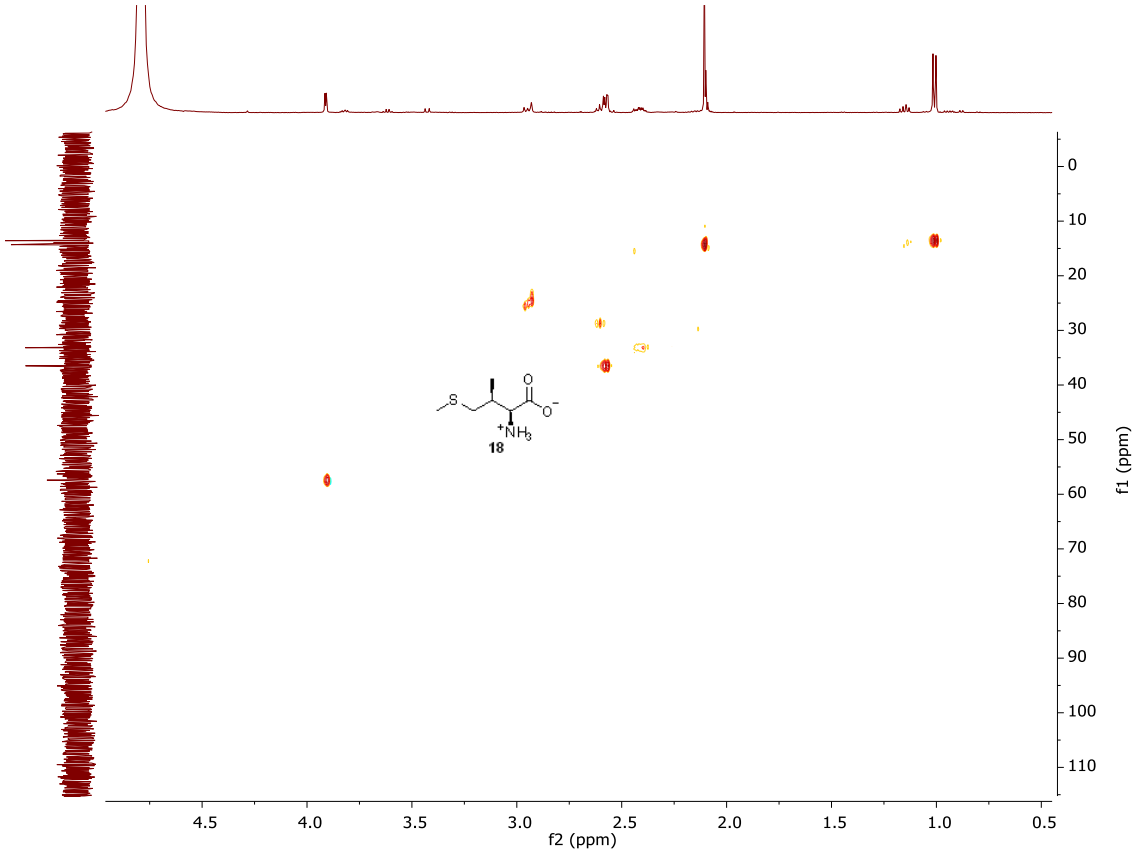
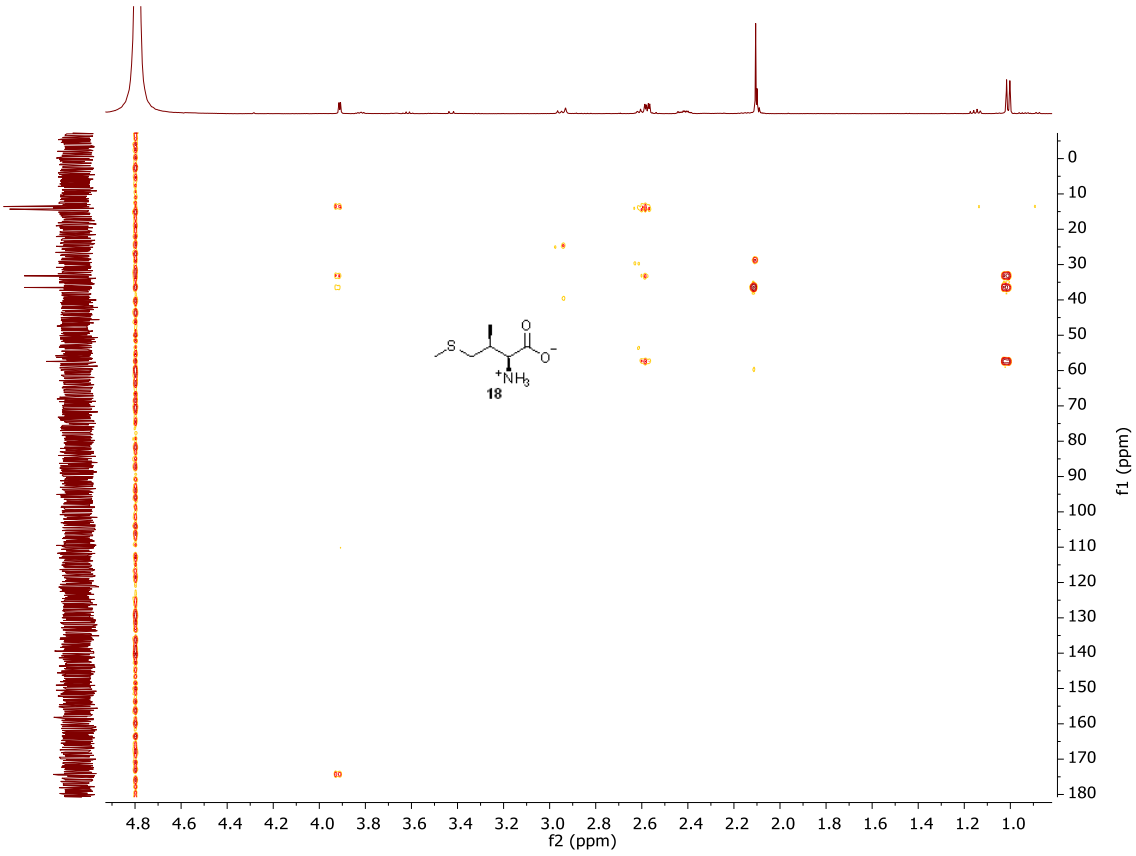
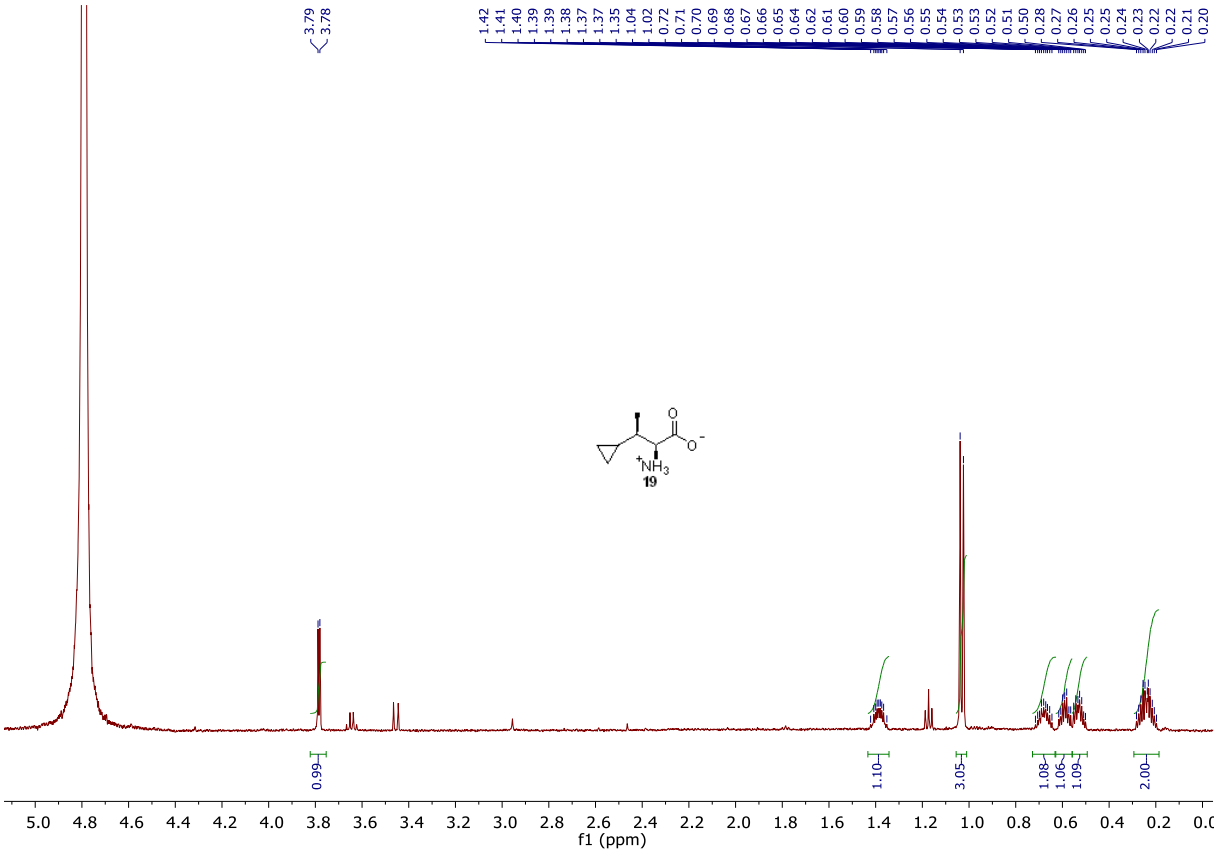
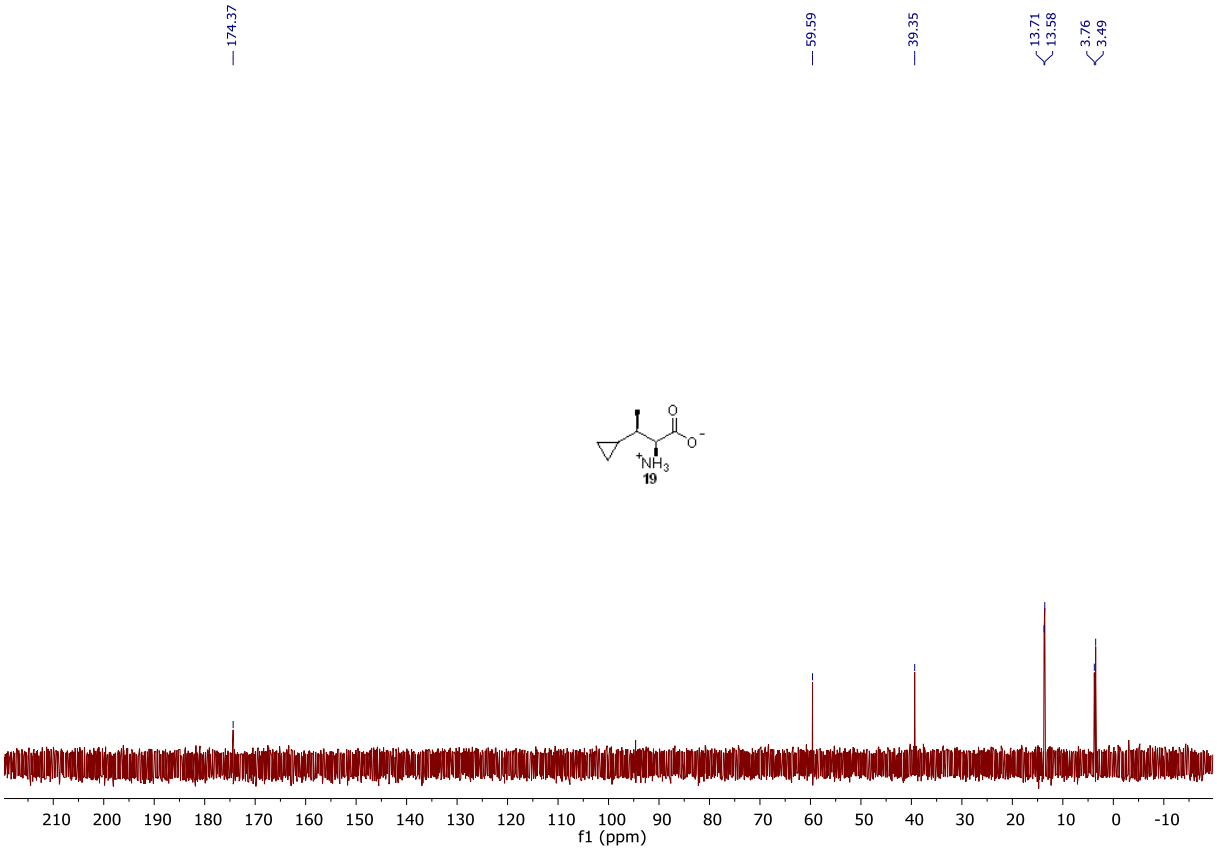
3.91
2.59
2.58
2.57
2.57
2.43
2.43
2.42
2.41
2.41
2.40
2.39
2.38
2.11
1.02
1.00
18
1.00
1.89
0.94
3.26
0.09
2.95
5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f1 (ppm)

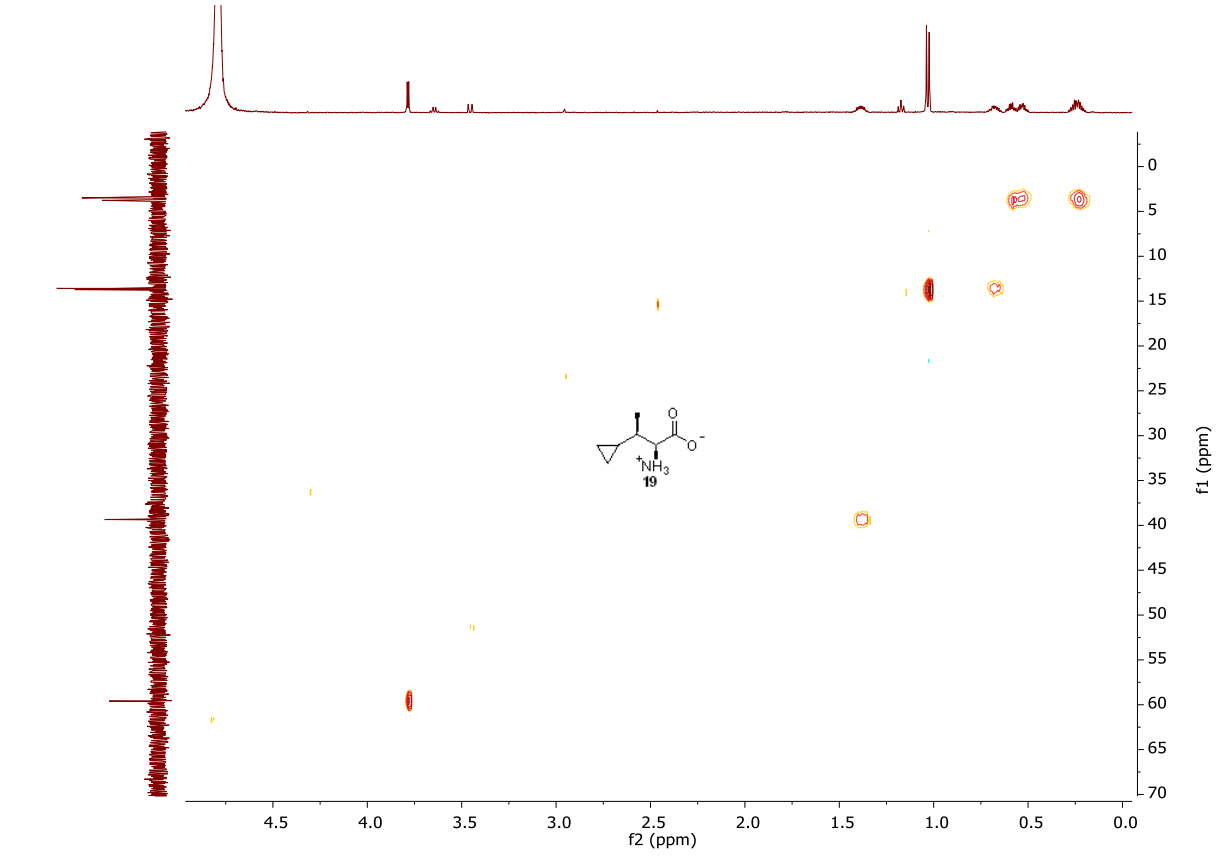

174.32
57.42
36.48
33.17
14.30
13.57
18
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

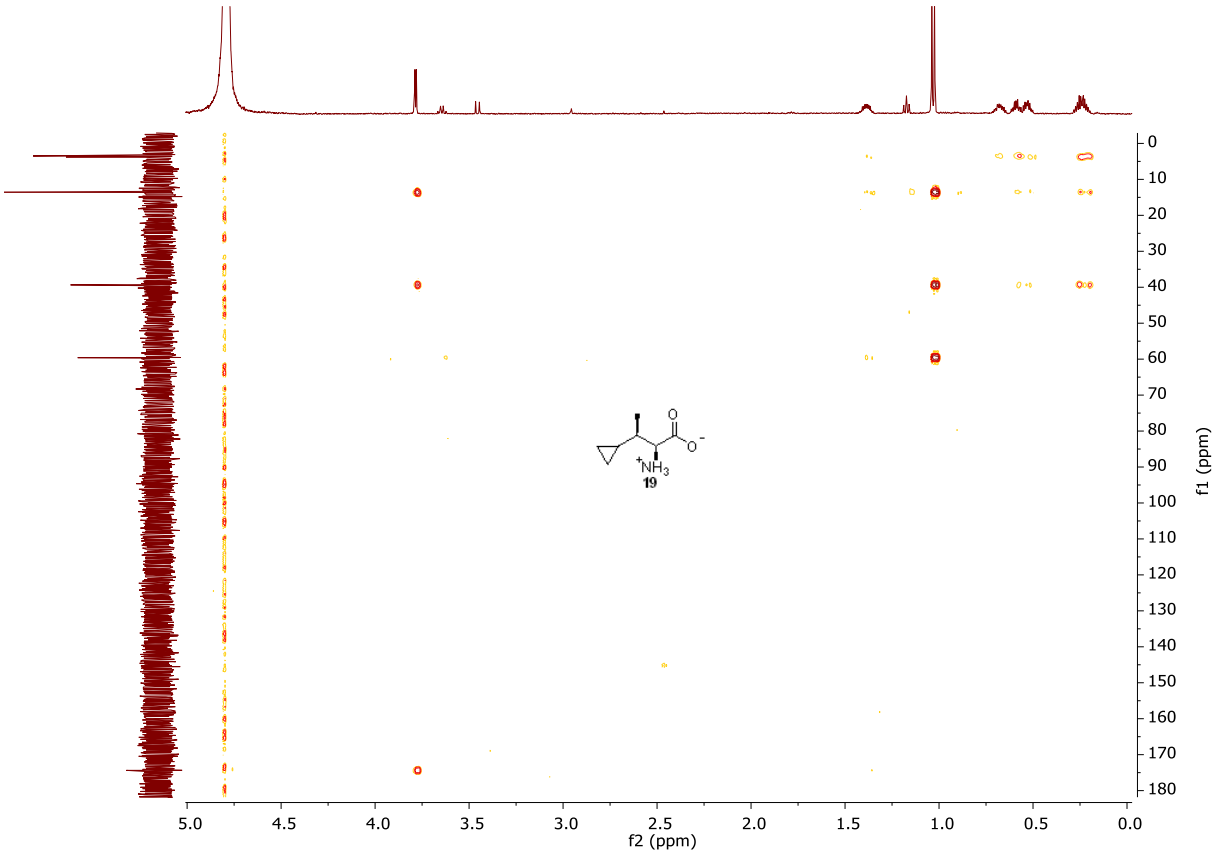

18
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
f1 (ppm)

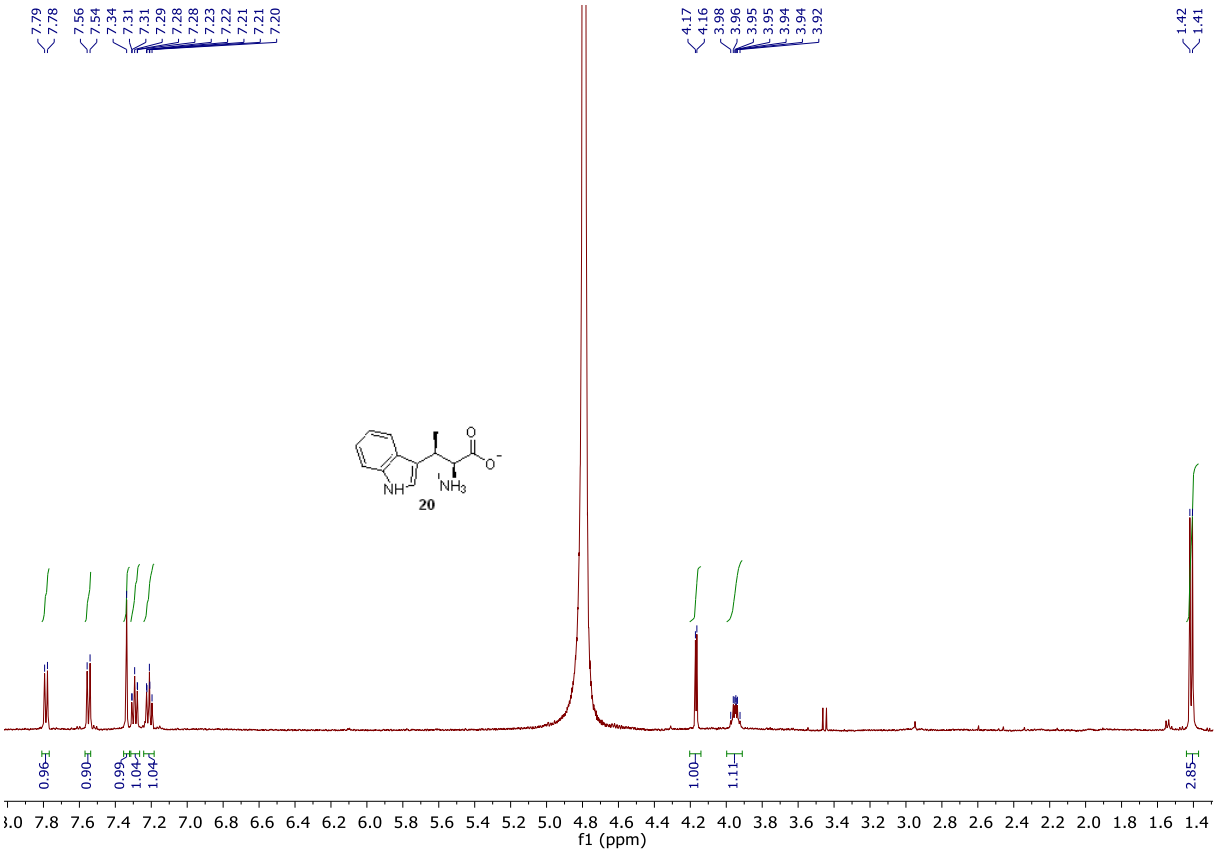

18
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)

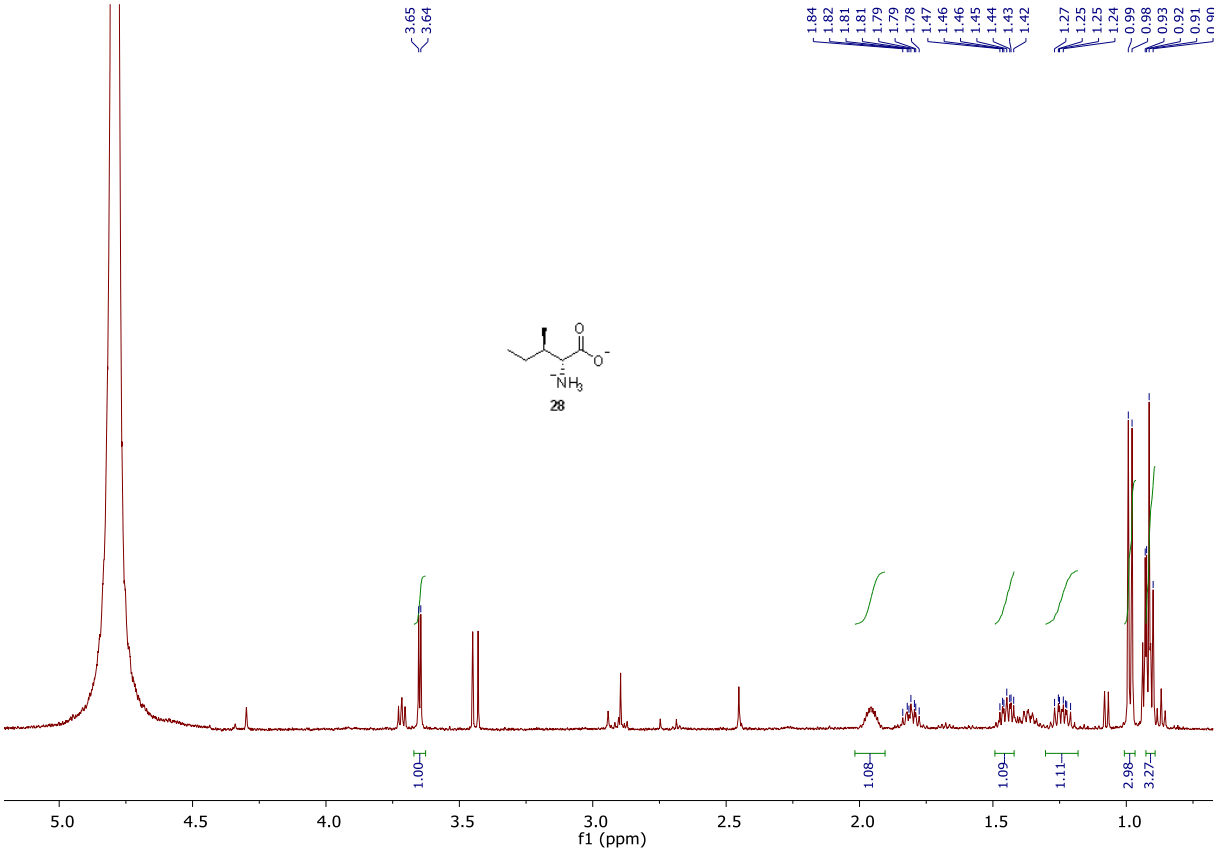

3.79
3.78
1.42
1.41
1.40
1.39
1.39
1.38
1.37
1.37
1.35
1.04
1.02
0.72
0.71
0.70
0.69
0.68
0.67
0.66
0.65
0.64
0.62
0.61
0.60
0.59
0.58
0.57
0.56
0.55
0.54
0.53
0.53
0.52
0.51
0.50
0.28
0.27
0.26
0.25
0.25
0.24
0.23
0.22
0.22
0.21
0.20
19
0.99
1.10
3.05
1.08
1.06
1.09
2.00
5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0
f1 (ppm)

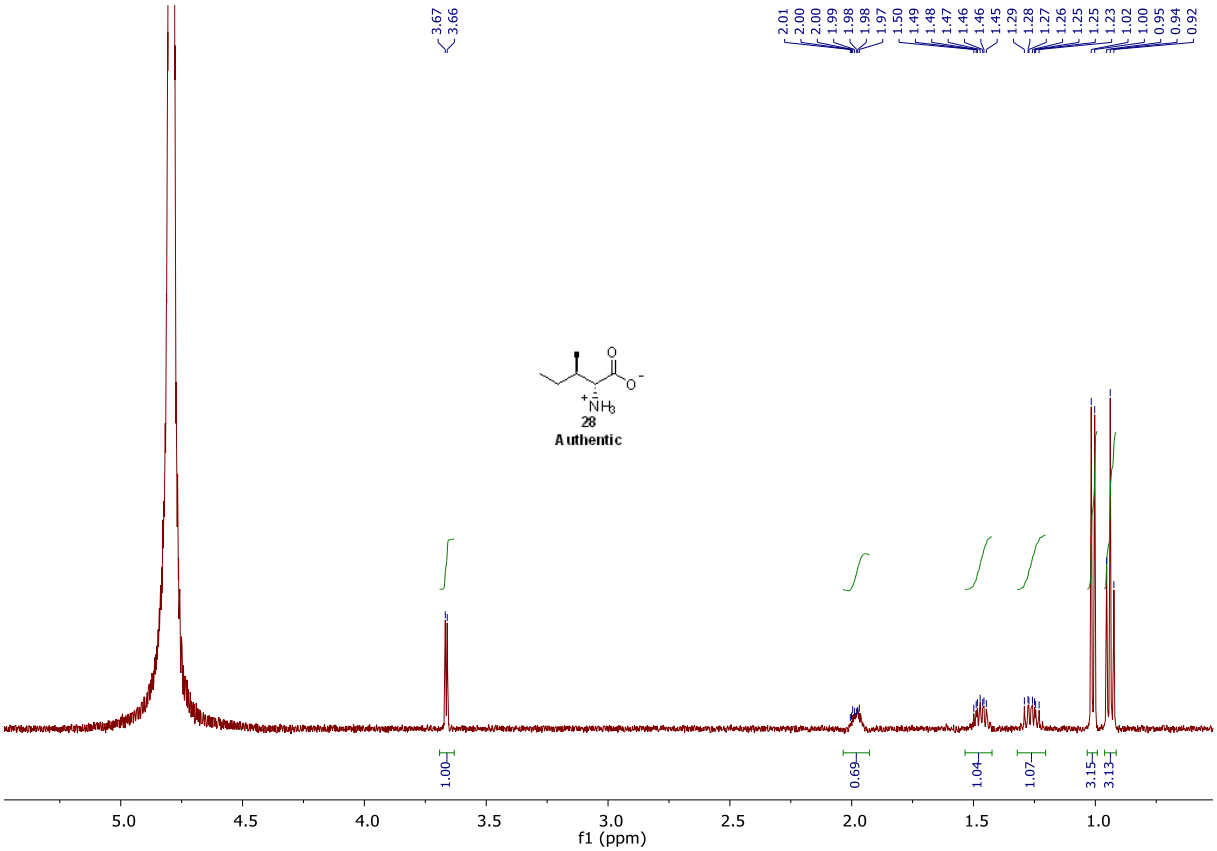

174.37
59.59
39.35
13.71
13.58
3.76
3.49
19
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


f1 (ppm)
f2 (ppm)
19


f1 (ppm)
f2 (ppm)
19


7.79
7.78
7.56
7.54
7.34
7.31
7.31
7.29
7.28
7.28
7.23
7.22
7.21
7.21
7.20
4.17
4.16
3.98
3.96
3.95
3.95
3.94
3.94
3.92
1.42
1.41
NH
NH3
O
O-
20
0.96
0.90
0.99
1.04
1.04
1.00
1.11
2.85
f1 (ppm)


3.65
3.64
1.84
1.82
1.81
1.81
1.79
1.79
1.78
1.47
1.46
1.46
1.45
1.44
1.43
1.42
1.27
1.25
1.25
1.24
0.99
0.98
0.93
0.92
0.91
NH3
O
O-
28
1.00
1.08
1.09
1.11
2.98
3.27
f1 (ppm)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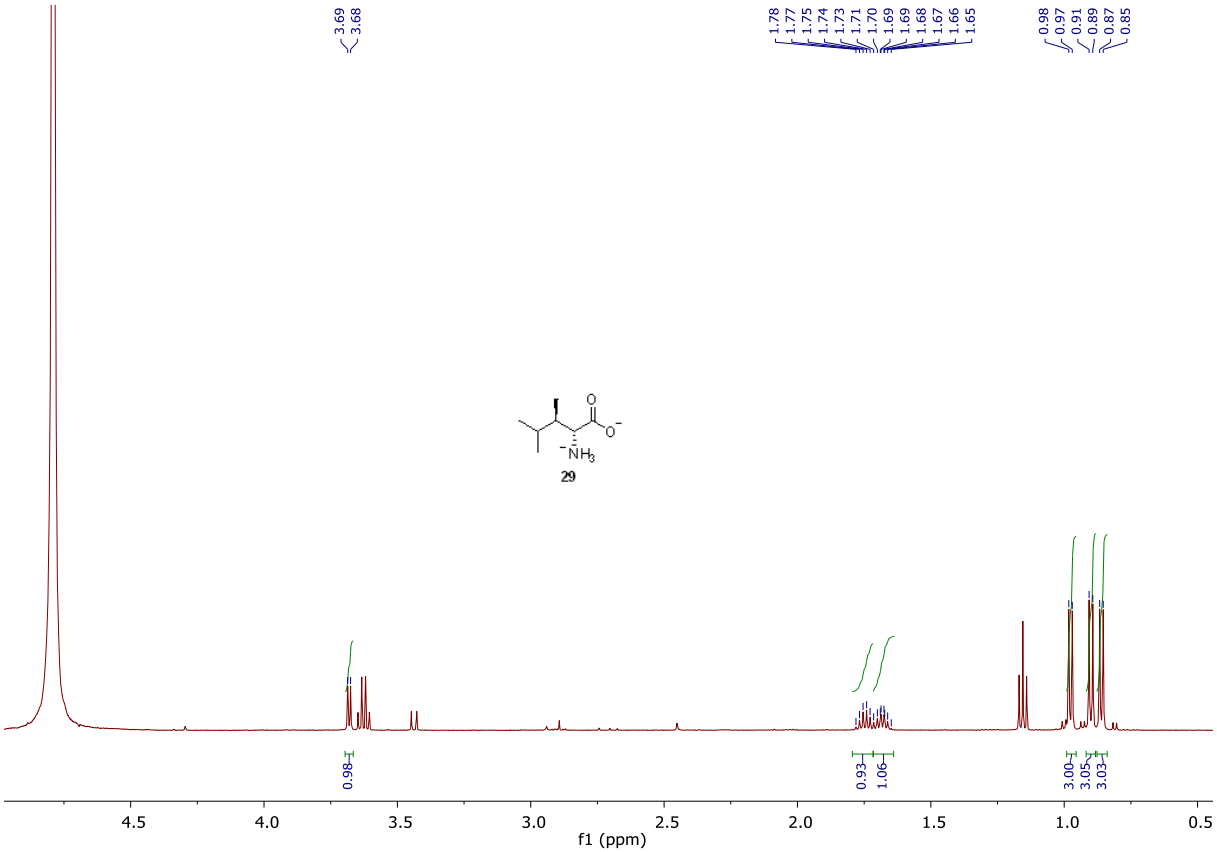
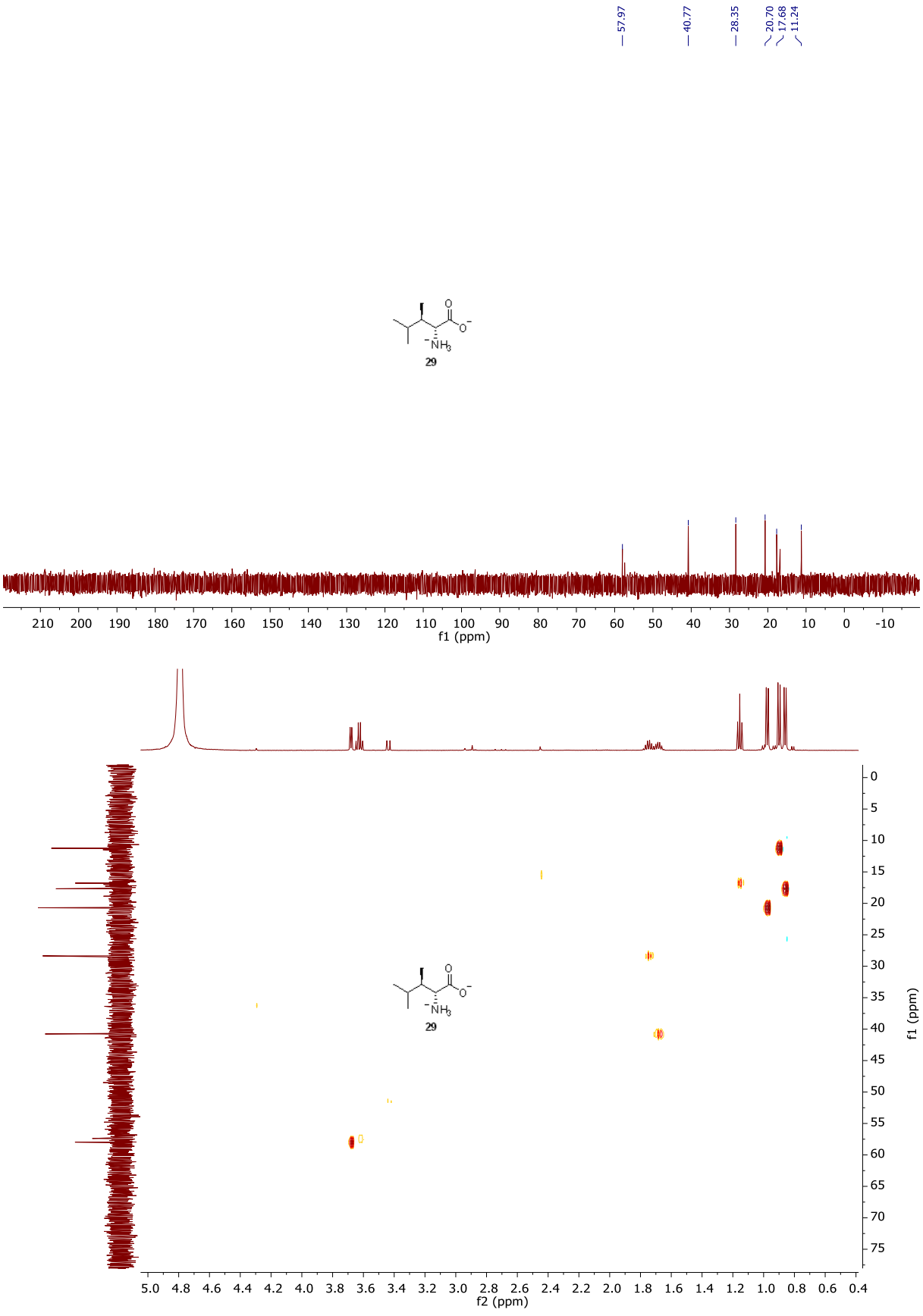
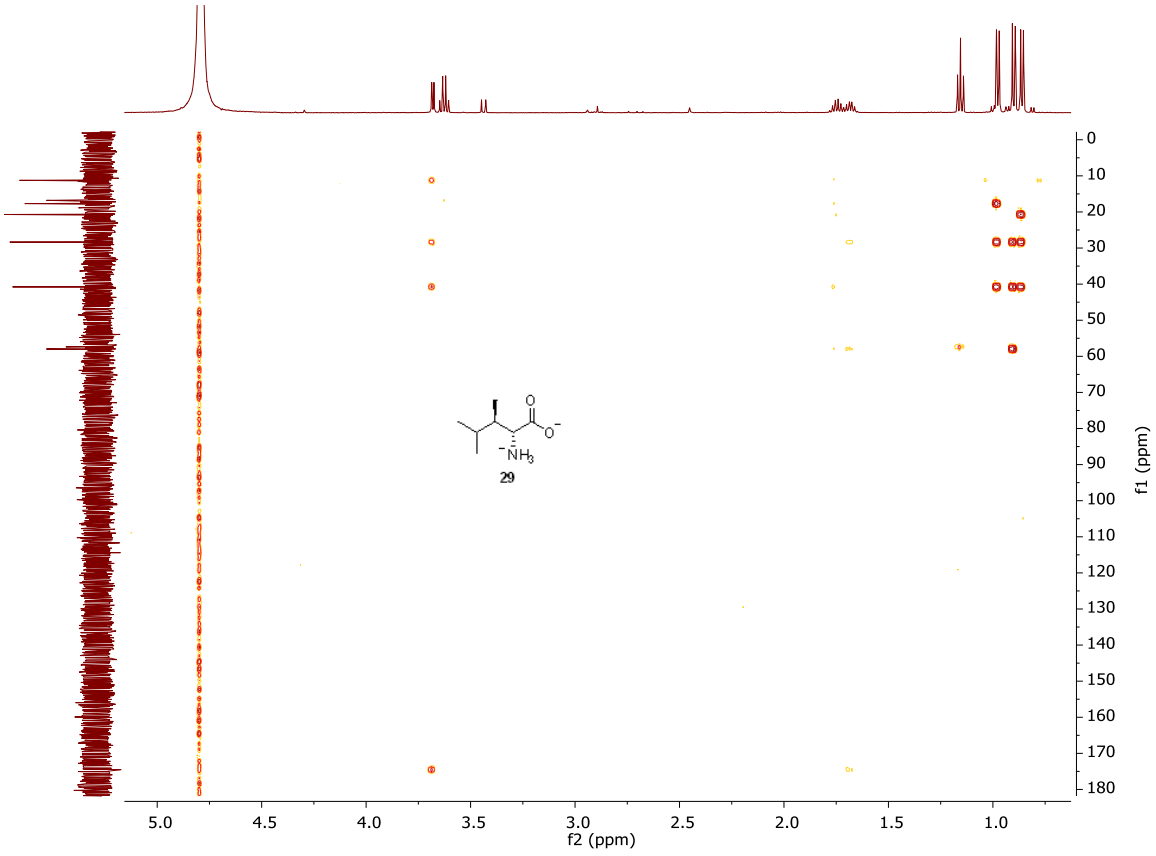
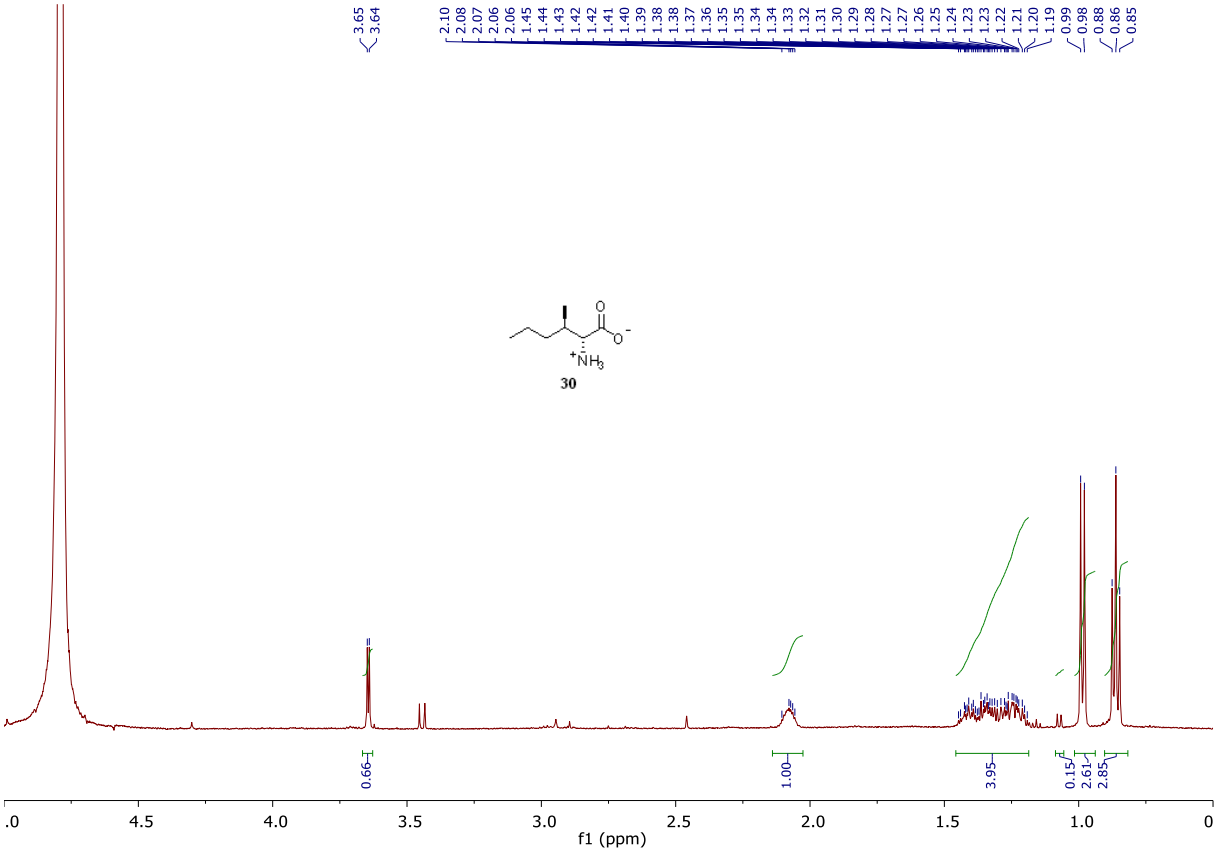
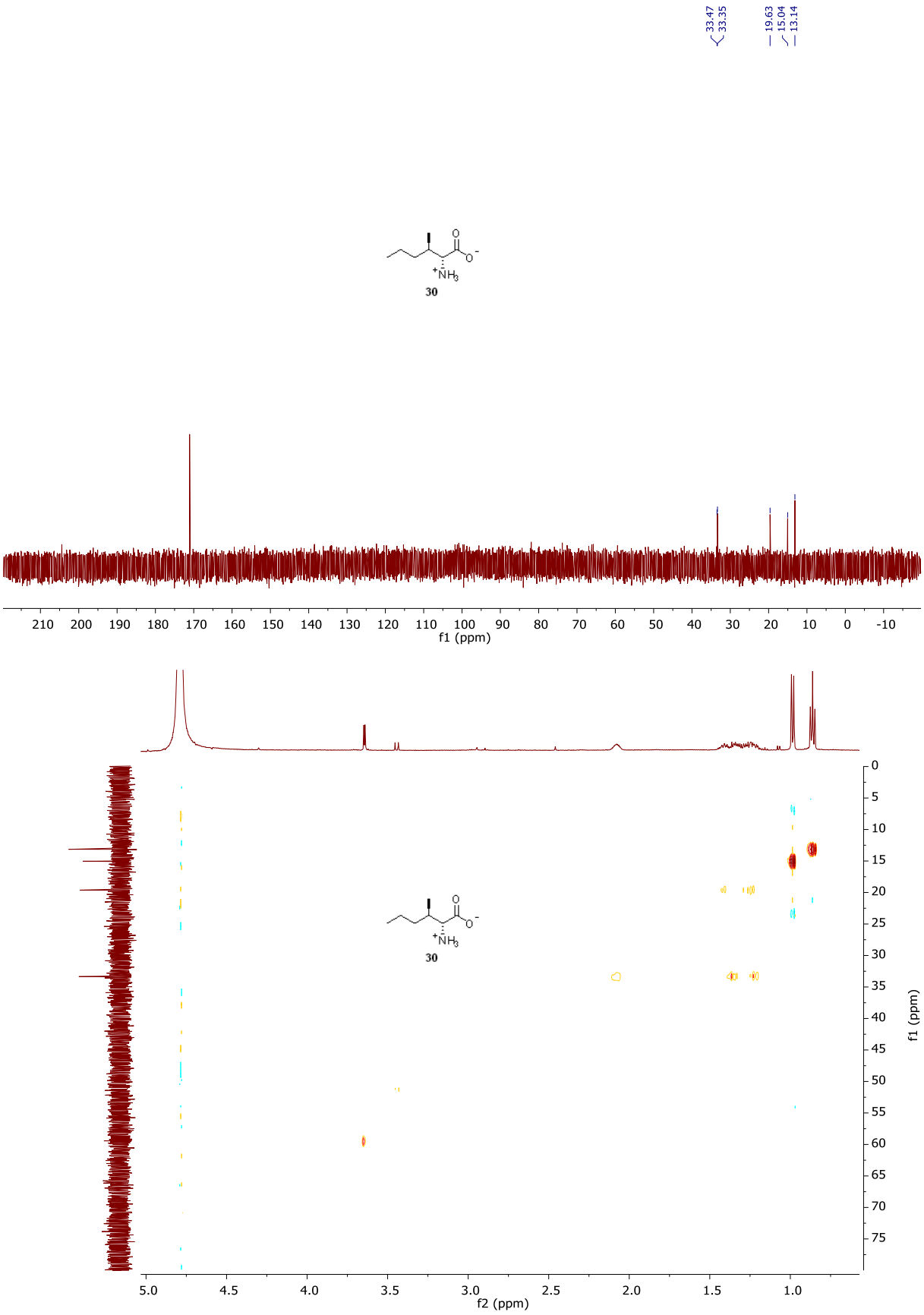
3.66
2.01
2.00
2.00
1.99
1.98
1.98
1.97
1.50
1.49
1.48
1.47
1.46
1.46
1.45
1.29
1.28
1.27
1.26
1.25
1.25
1.23
1.02
1.00
0.95
0.94
0.92
28
Authentic
1.00
0.69
1.04
1.07
3.15
3.13
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

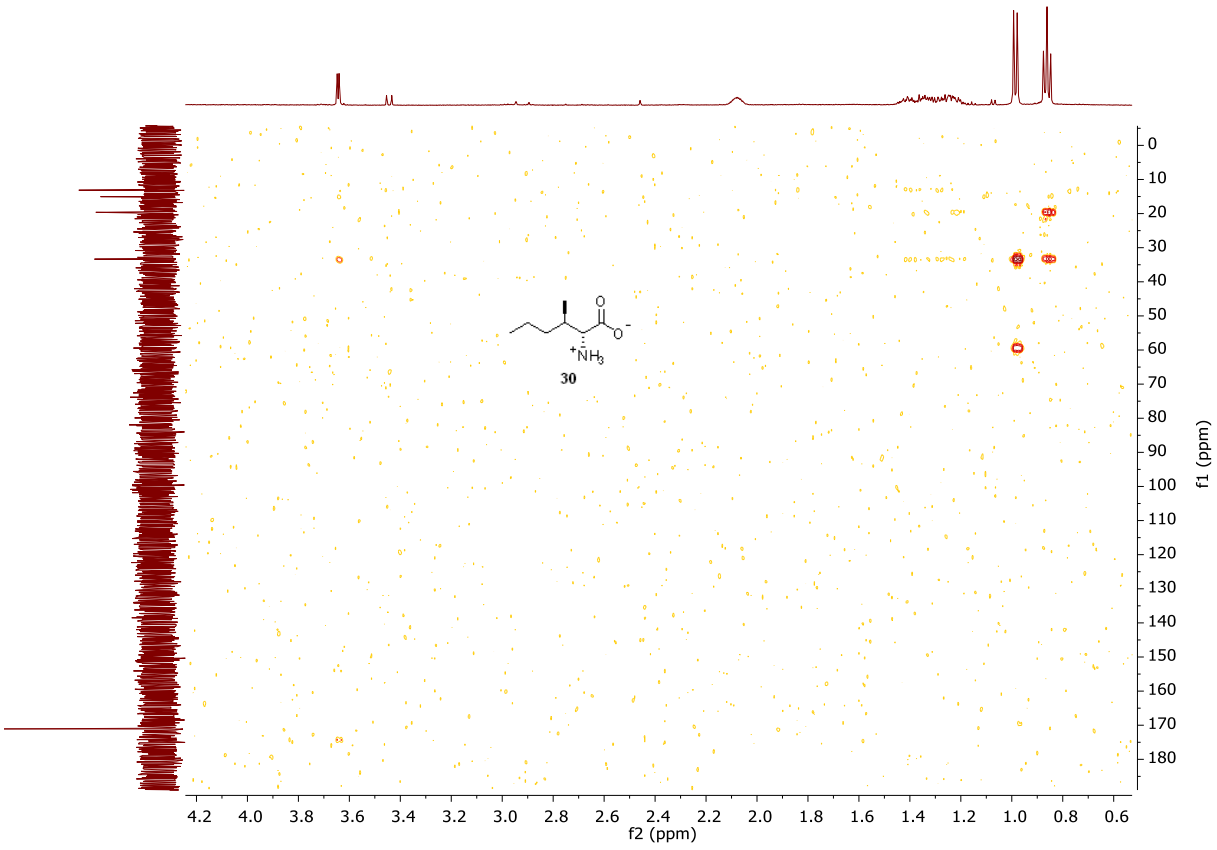

3.69
3.68
1.78
1.77
1.75
1.74
1.73
1.71
1.70
1.69
1.69
1.68
1.67
1.66
1.65
0.98
0.97
0.91
0.89
0.87
0.85
29
0.98
0.93
1.06
3.00
3.05
3.03
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

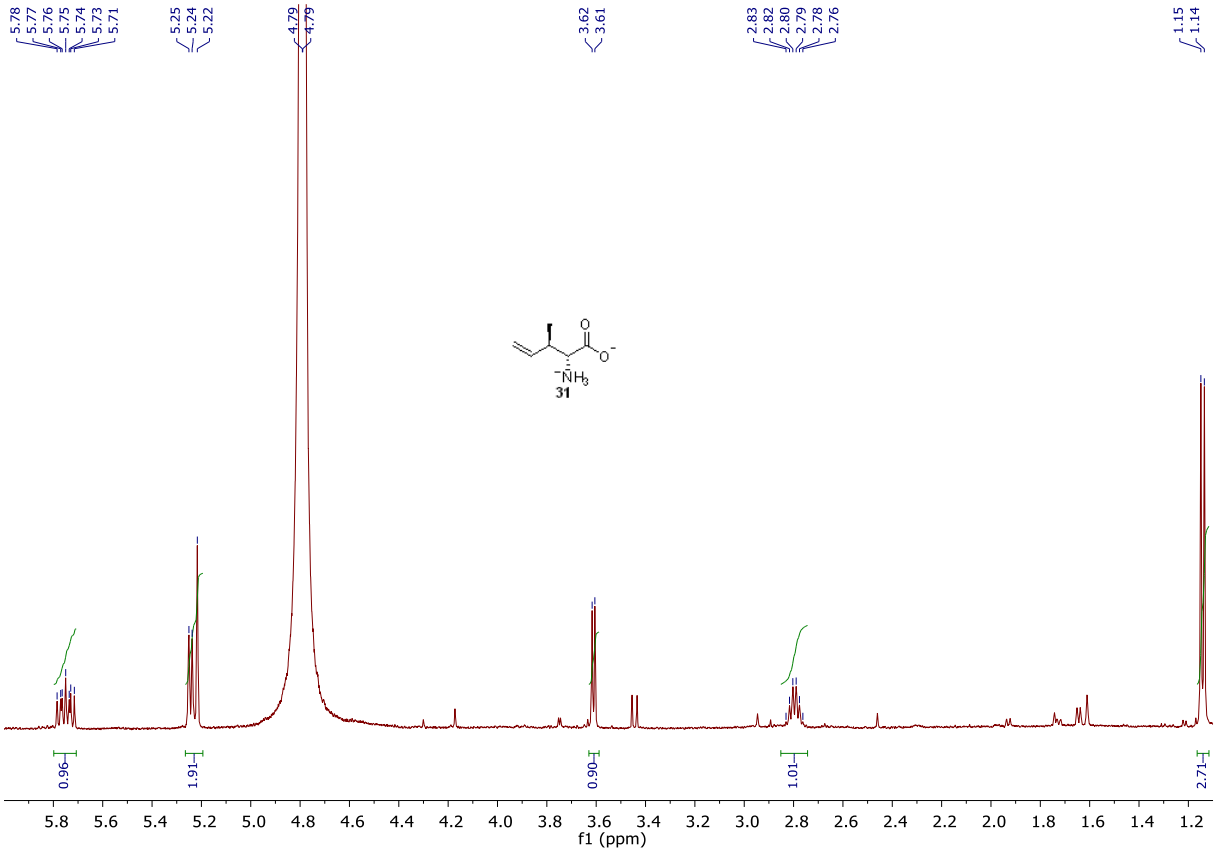

57.97
40.77
28.35
20.70
17.68
11.24
29
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
29
5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4
f2 (ppm)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
f1 (ppm)

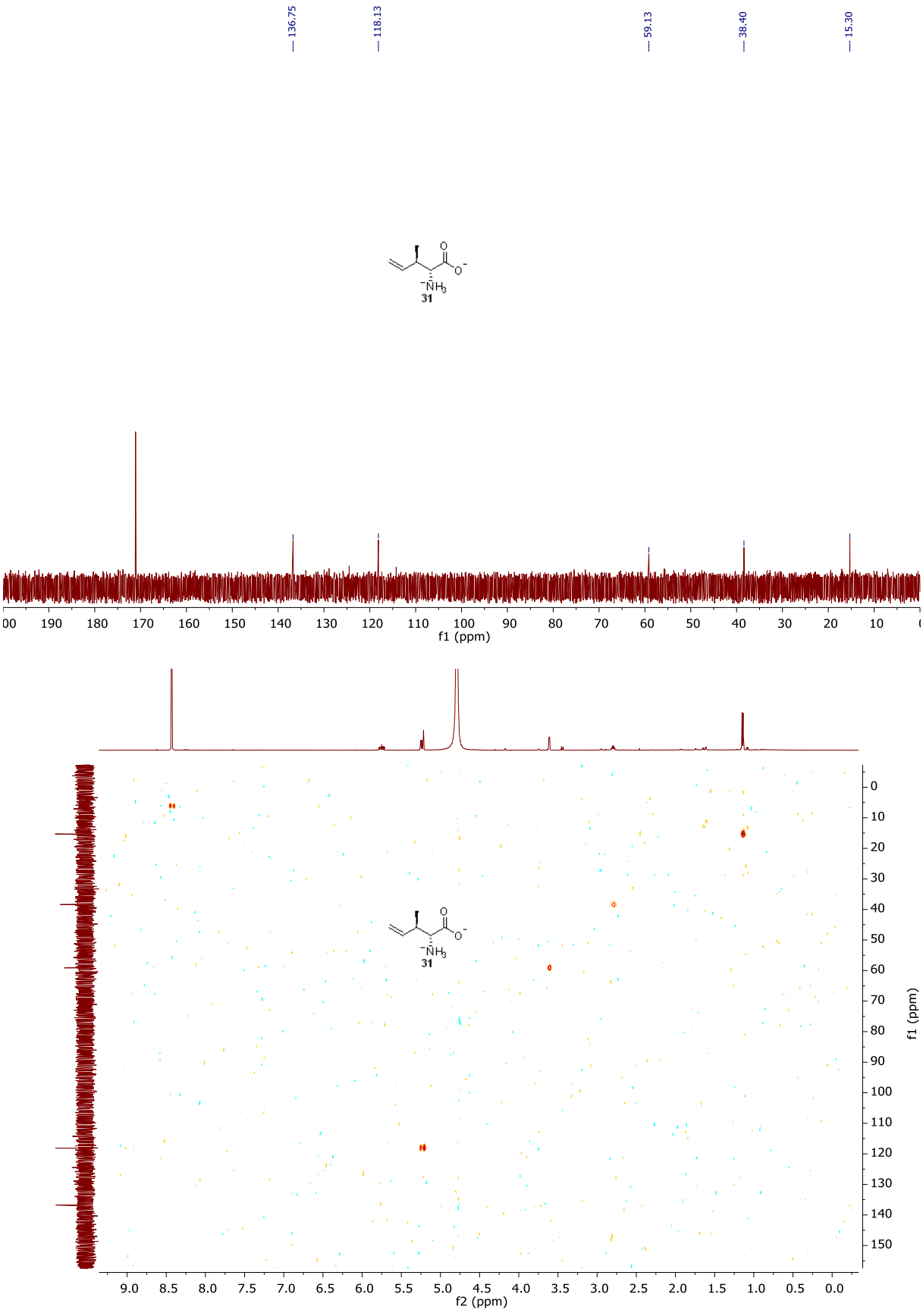

f2 (ppm)
f1 (ppm)
29

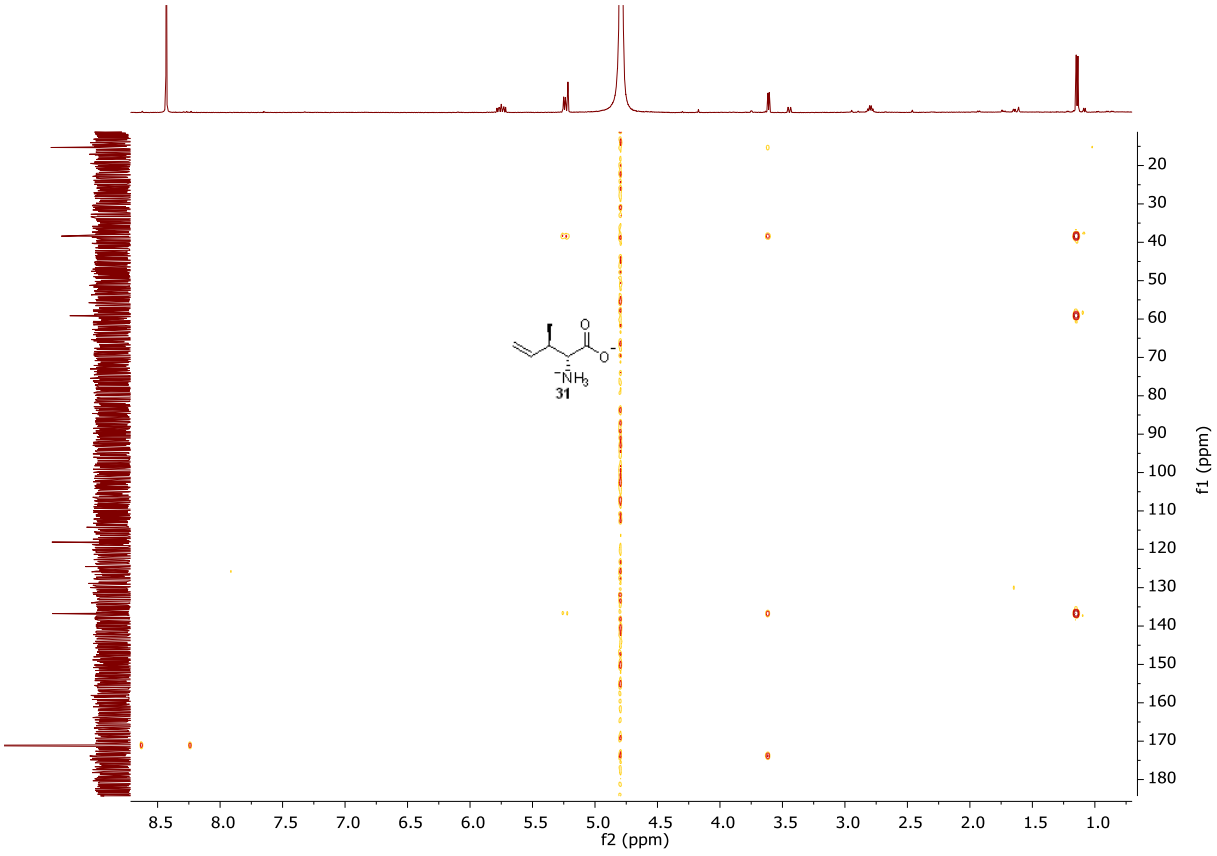

3.65
3.64
2.10
2.08
2.07
2.06
2.06
1.45
1.44
1.43
1.42
1.42
1.41
1.40
1.39
1.38
1.38
1.37
1.36
1.35
1.35
1.34
1.34
1.33
1.32
1.31
1.30
1.29
1.28
1.27
1.27
1.26
1.25
1.24
1.23
1.23
1.22
1.21
1.20
1.19
0.99
0.98
0.88
0.86
0.85
0.66
1.00
3.95
0.15
2.61
2.85
f1 (ppm)
30

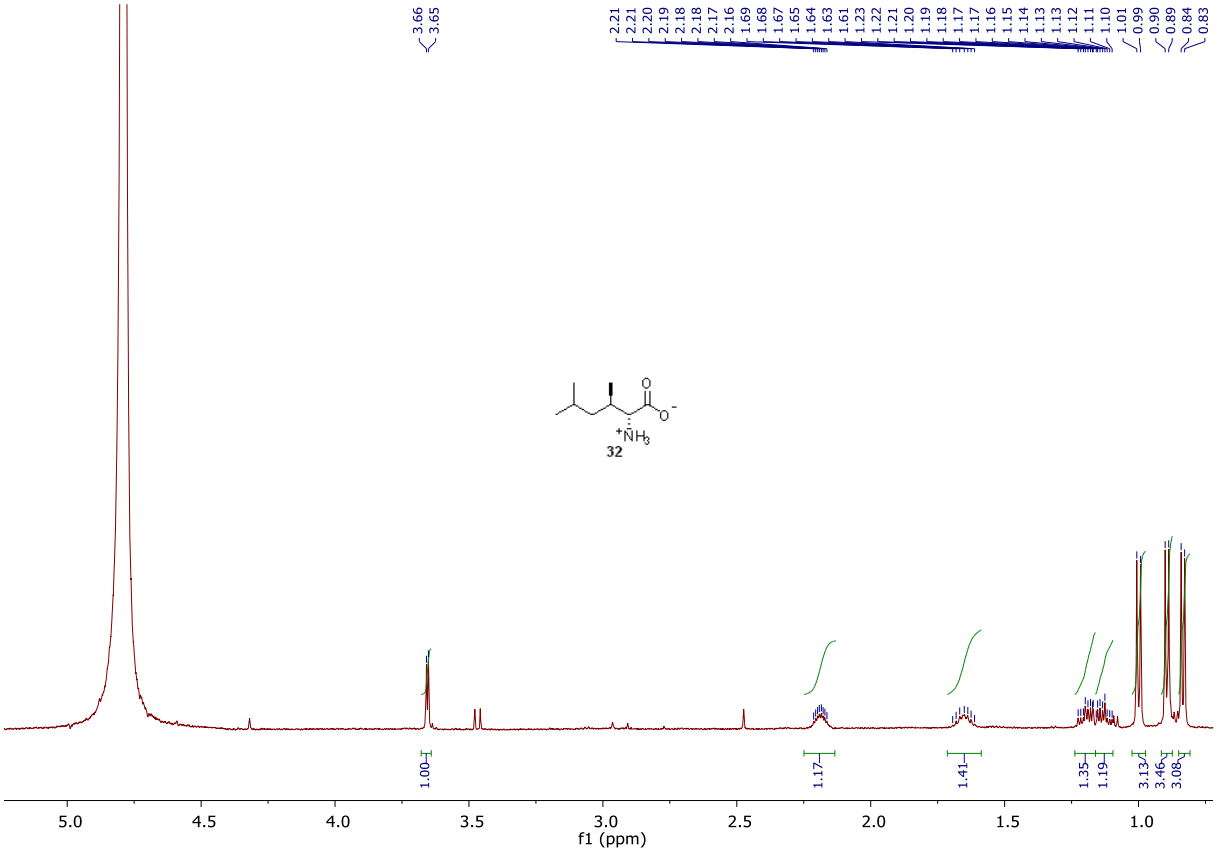

33.47
33.35
19.63
15.04
13.14
30
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
30
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f2 (ppm)
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
f1 (ppm)

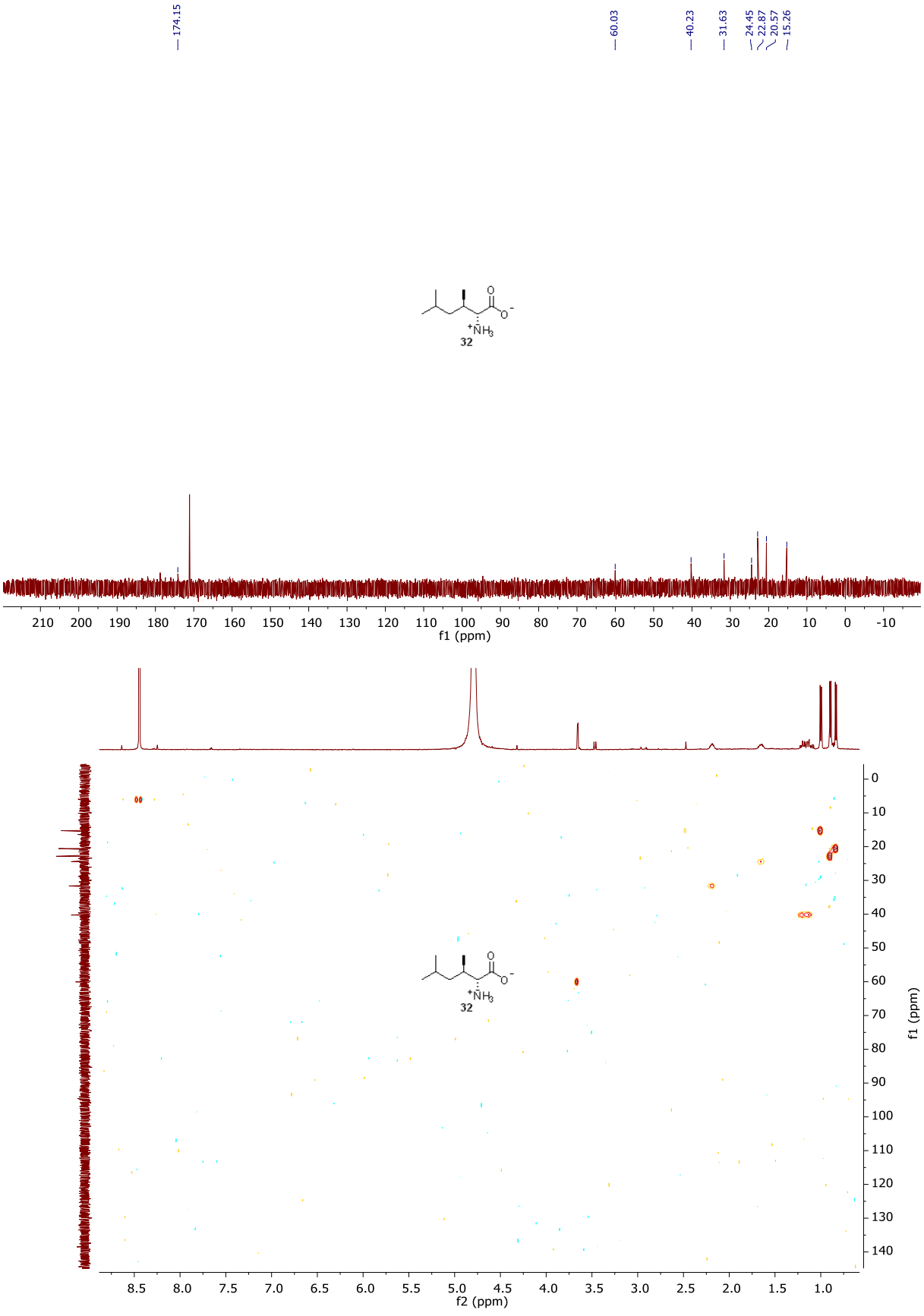

30
f2 (ppm)
f1 (ppm)


5.78
5.77
5.76
5.75
5.74
5.73
5.71
5.25
5.24
5.22
4.79
4.79
3.62
3.61
2.83
2.82
2.80
2.79
2.78
2.76
1.15
1.14
31
0.96
1.91
0.90
1.01
2.71
f1 (ppm)


136.75
118.13
59.13
38.40
15.30
31
f1 (ppm)
31
f1 (ppm)
f2 (ppm)


31
f2 (ppm)
f1 (ppm)


32
f1 (ppm)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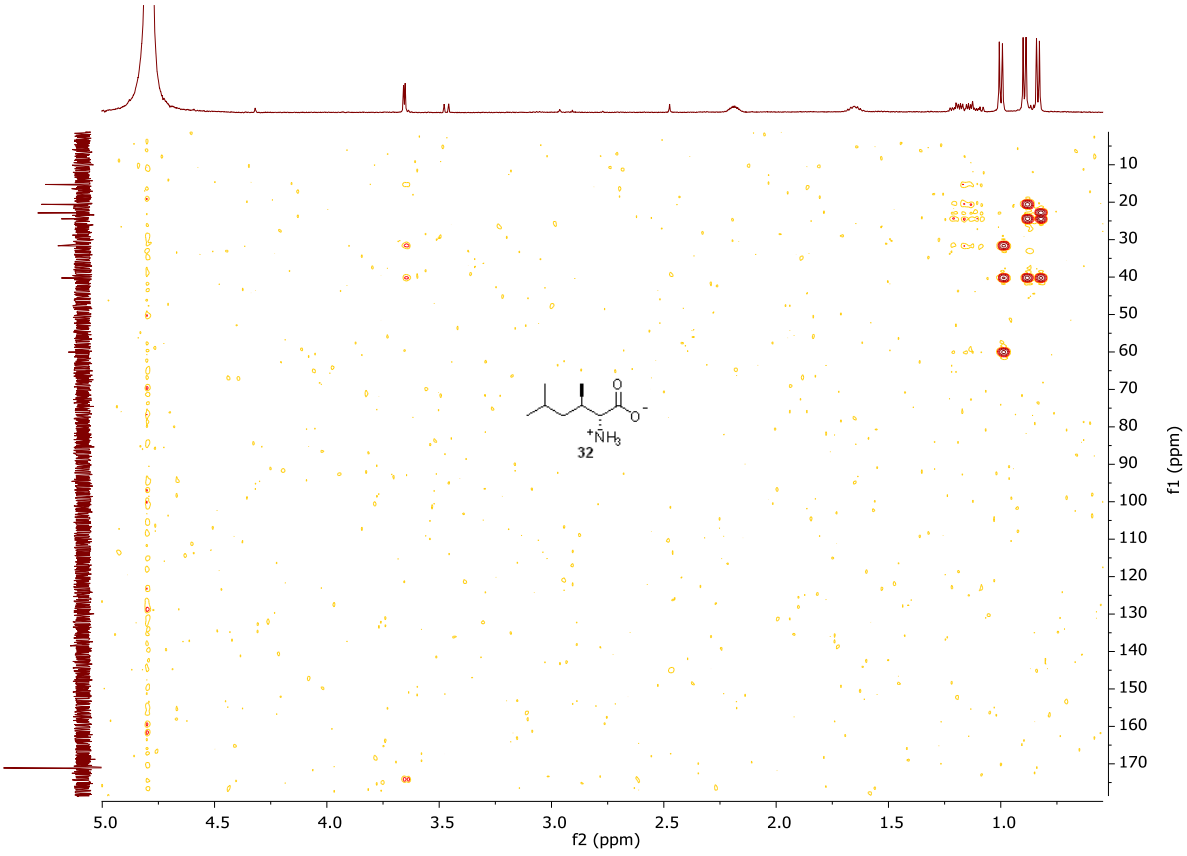
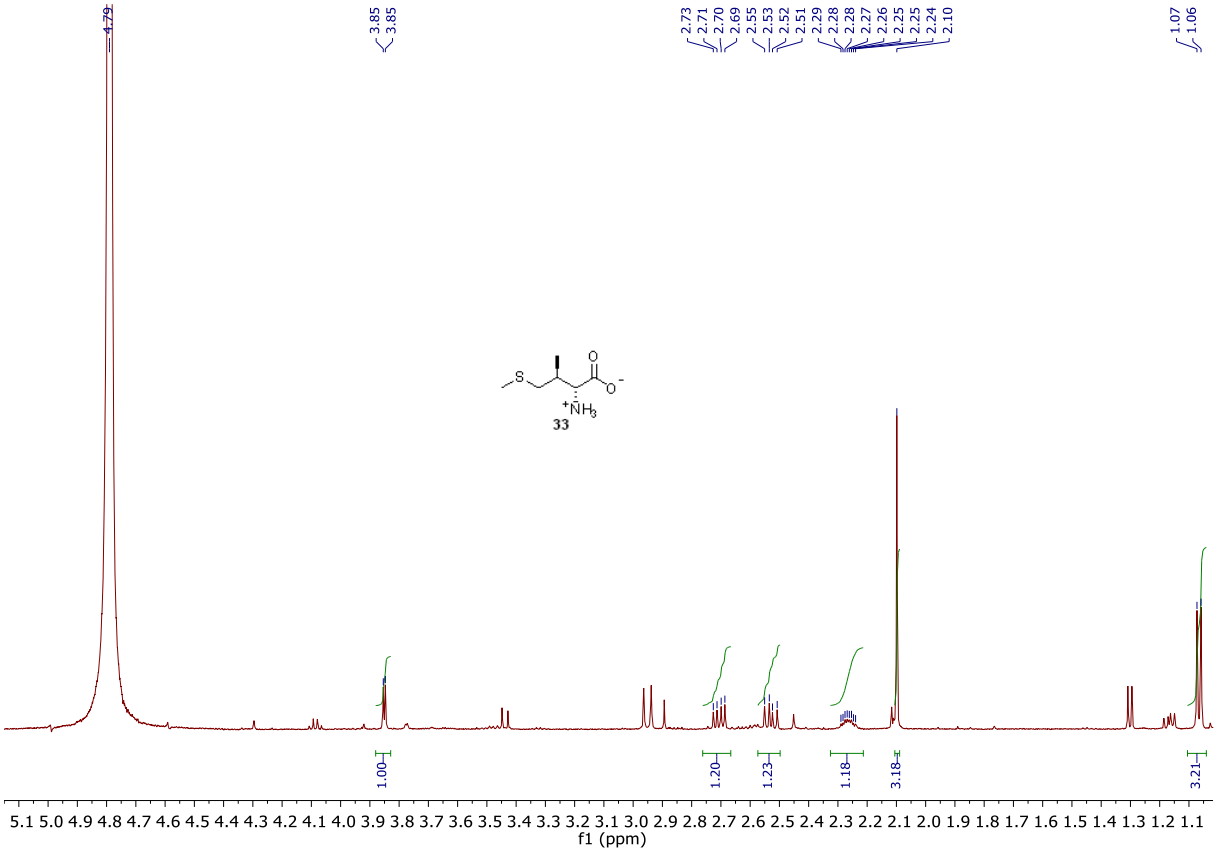
40.23
31.63
24.45
22.87
20.57
15.26
32
f1 (ppm)
f2 (ppm)


f2 (ppm)
f1 (ppm)
32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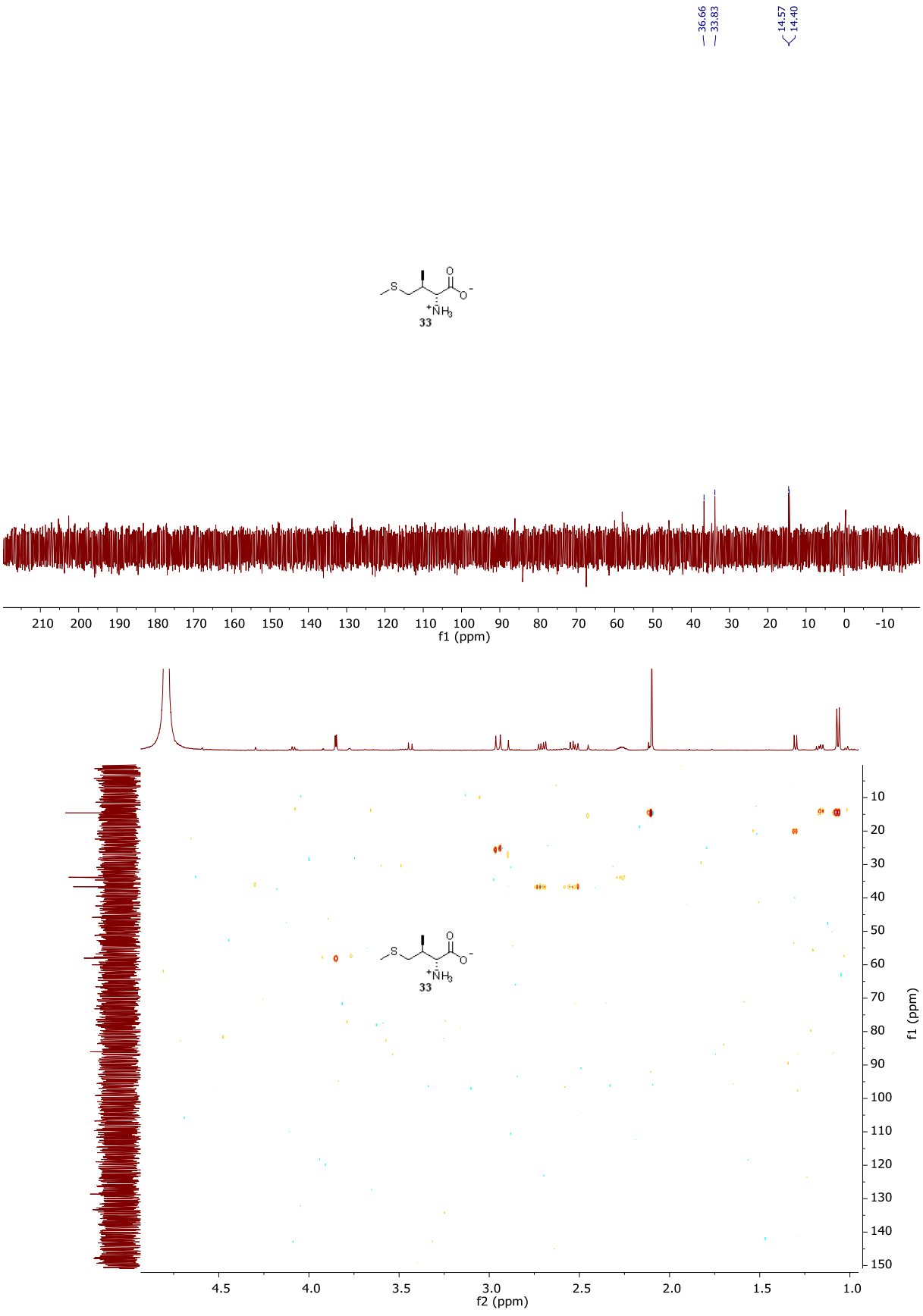
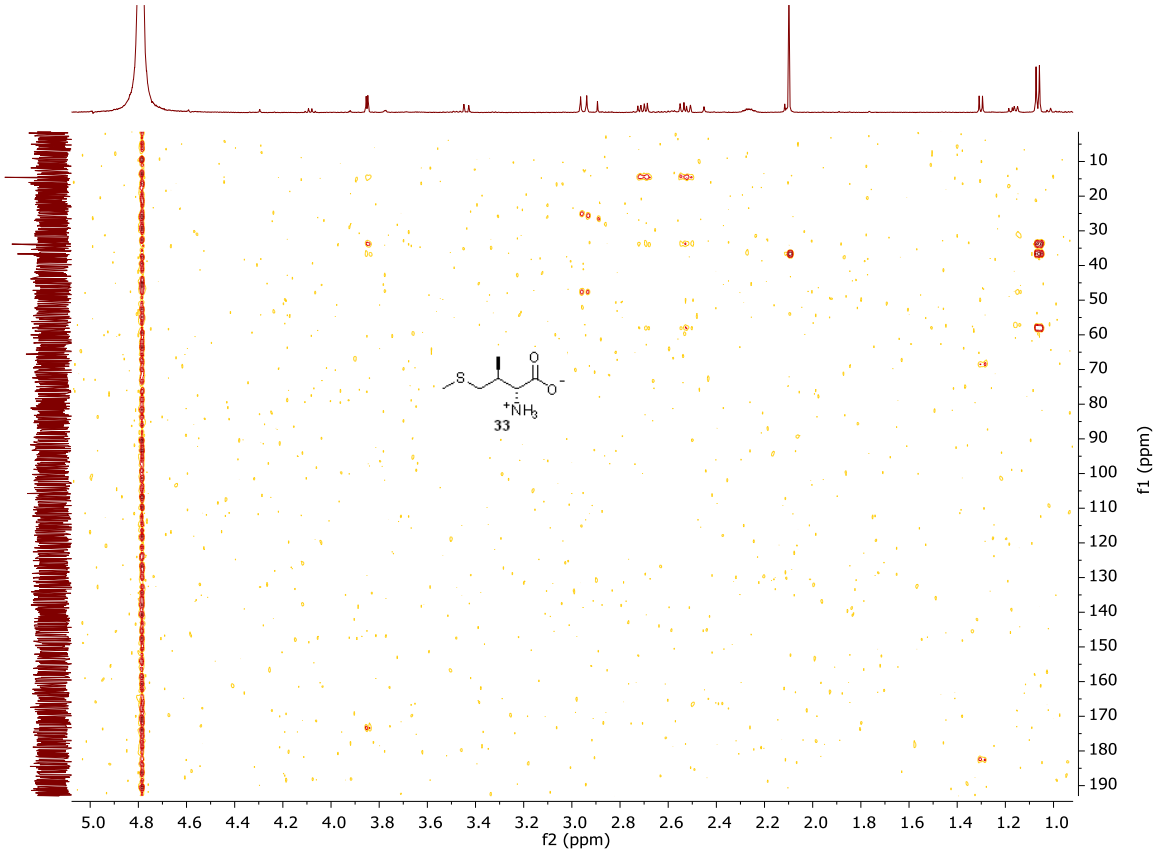
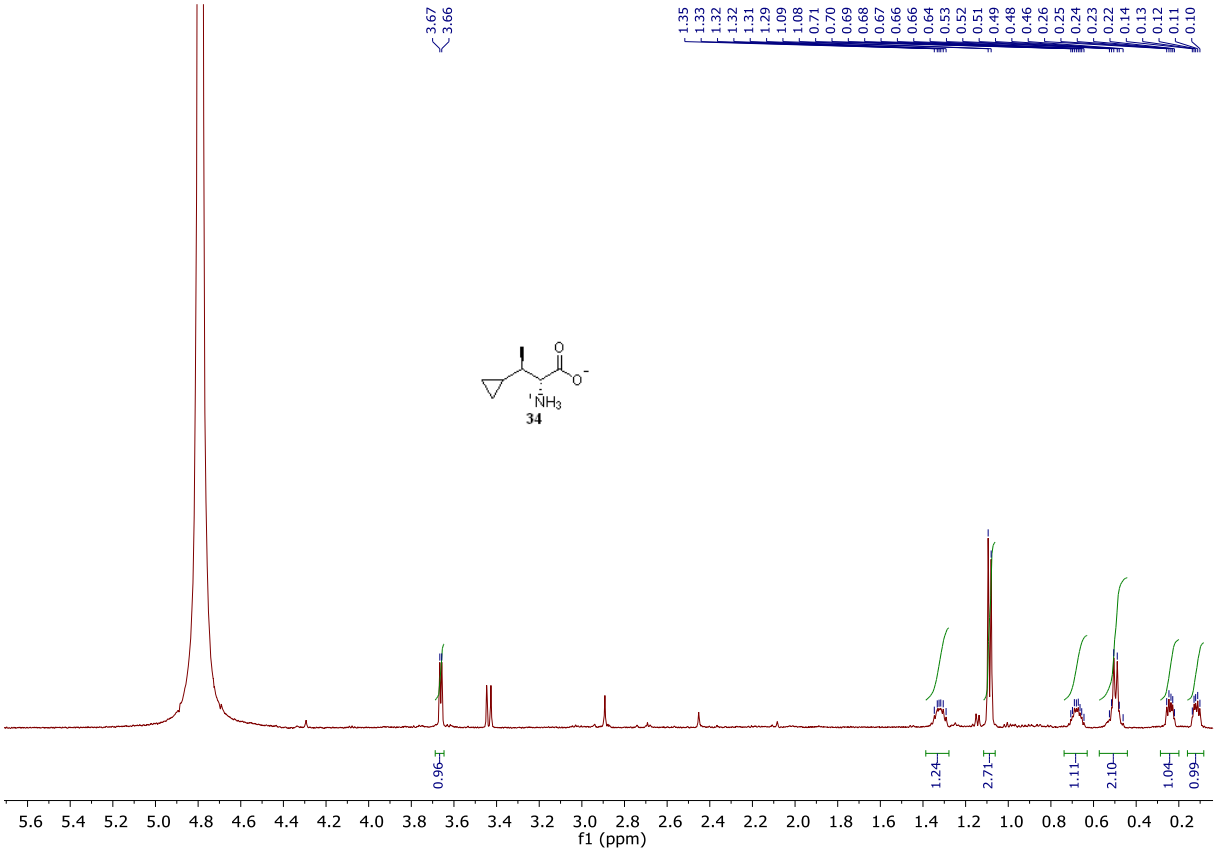
3.85
2.73
2.71
2.70
2.69
2.55
2.53
2.52
2.51
2.29
2.28
2.28
2.27
2.26
2.25
2.25
2.24
2.10
1.07
1.06
33
1.00
1.20
1.23
1.18
3.18
3.21
f1 (ppm)


36.66
33.83
14.57
14.40
33
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f2 (ppm)
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
f1 (ppm)


33
f2 (ppm)
f1 (ppm)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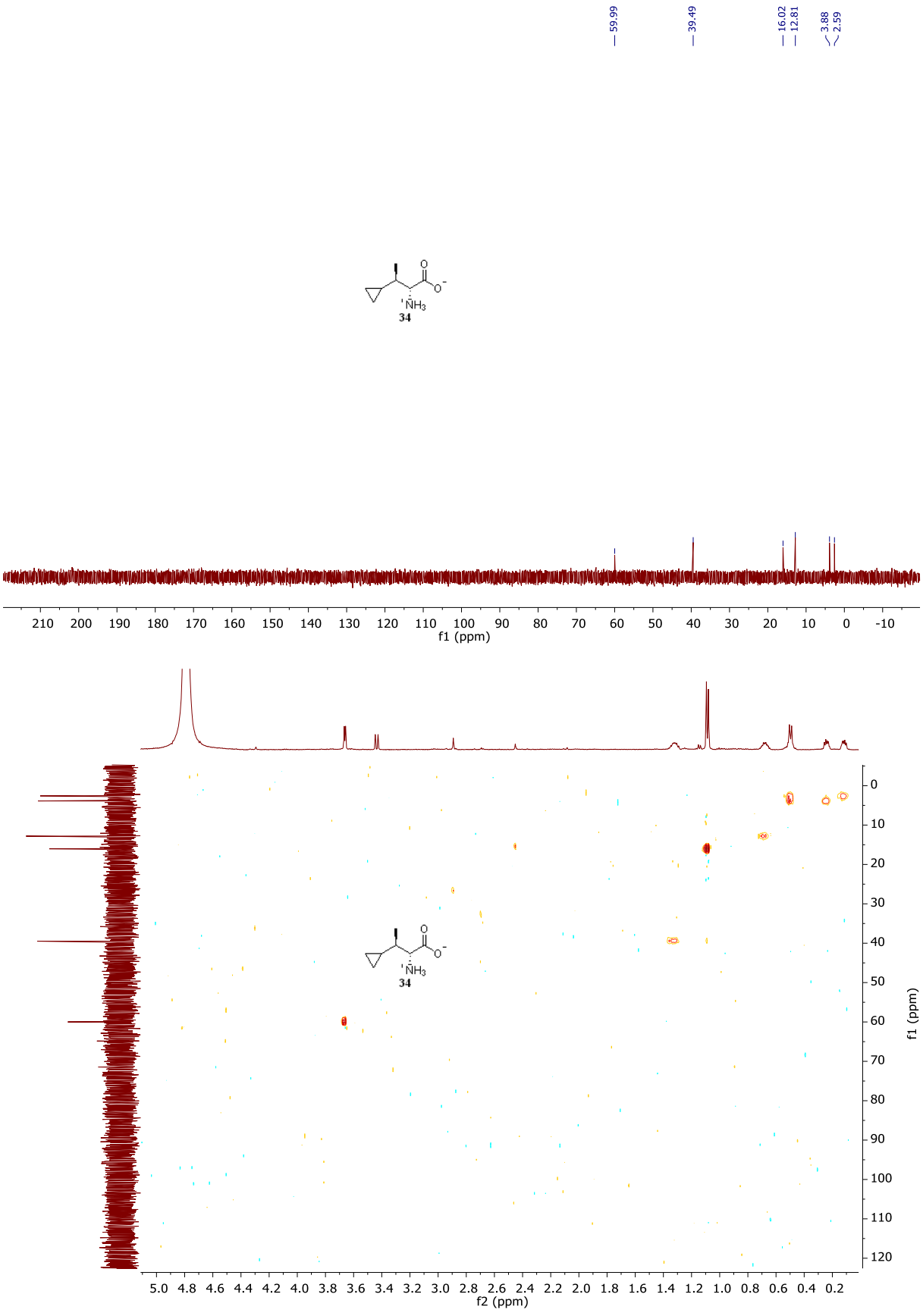
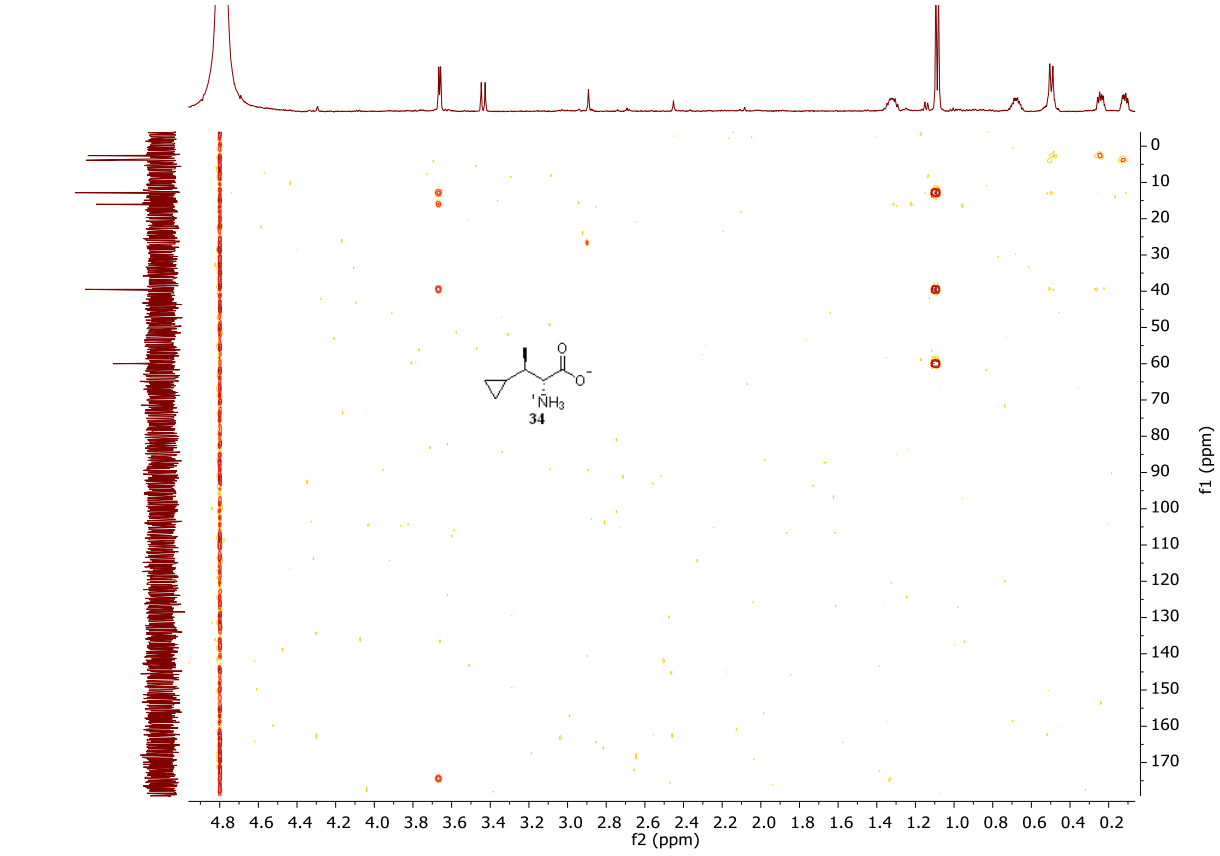
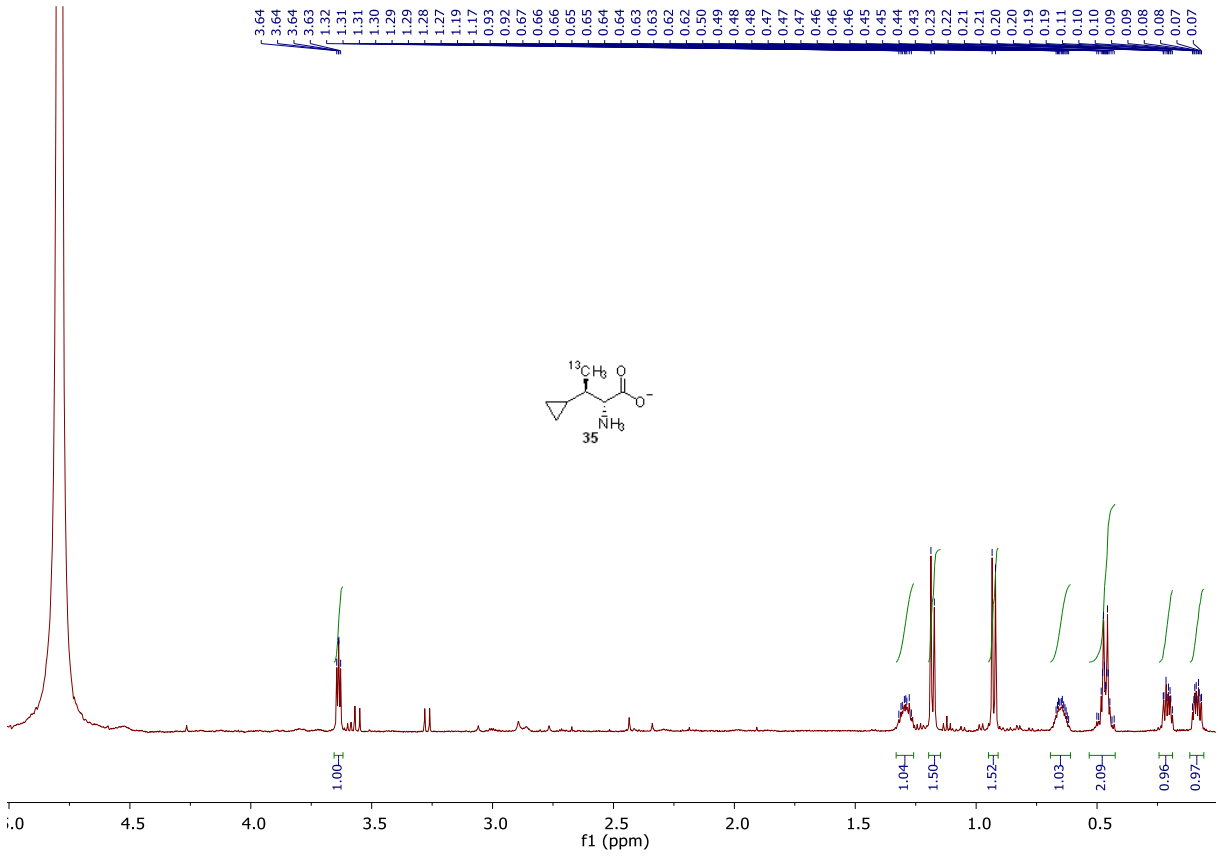
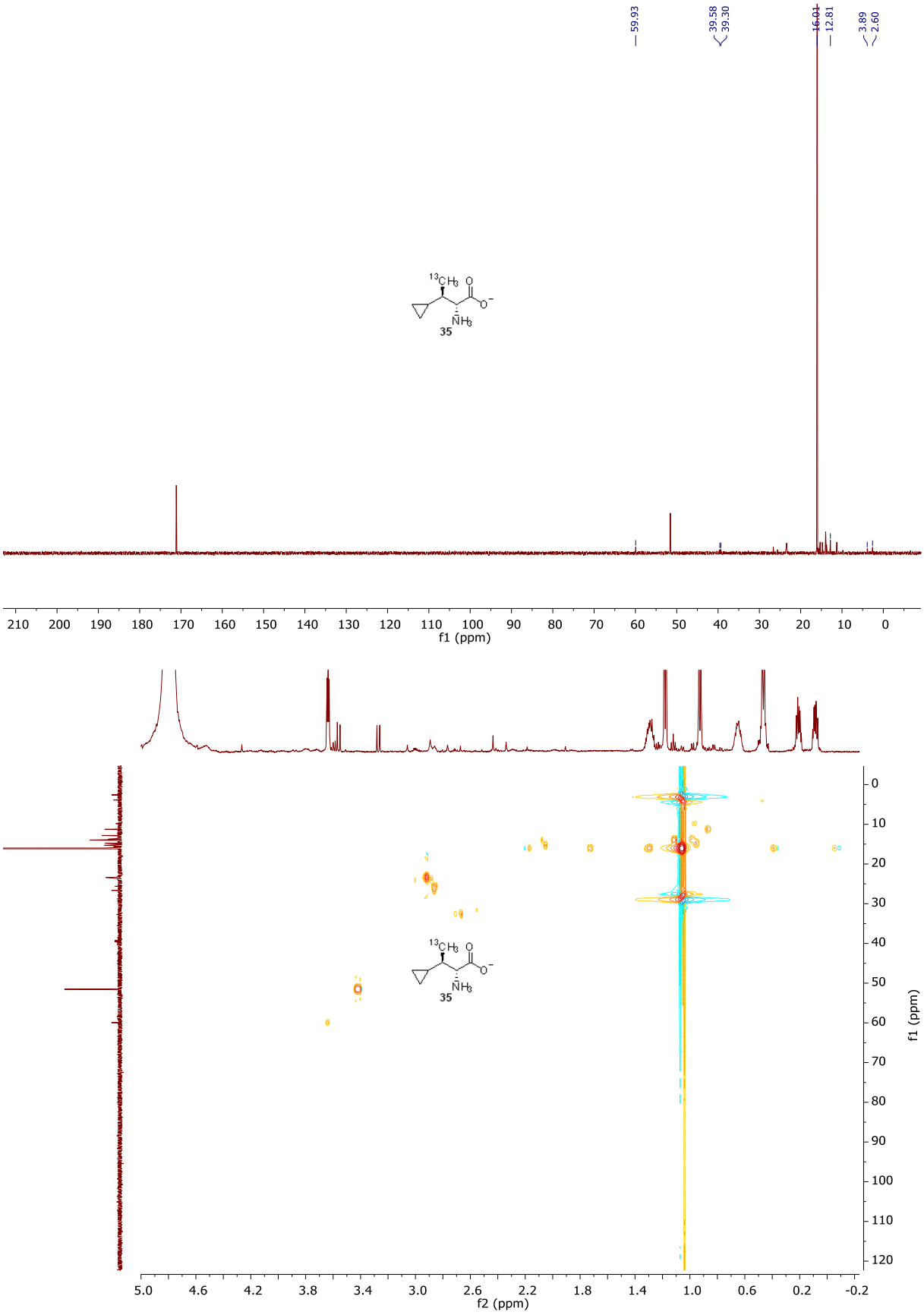
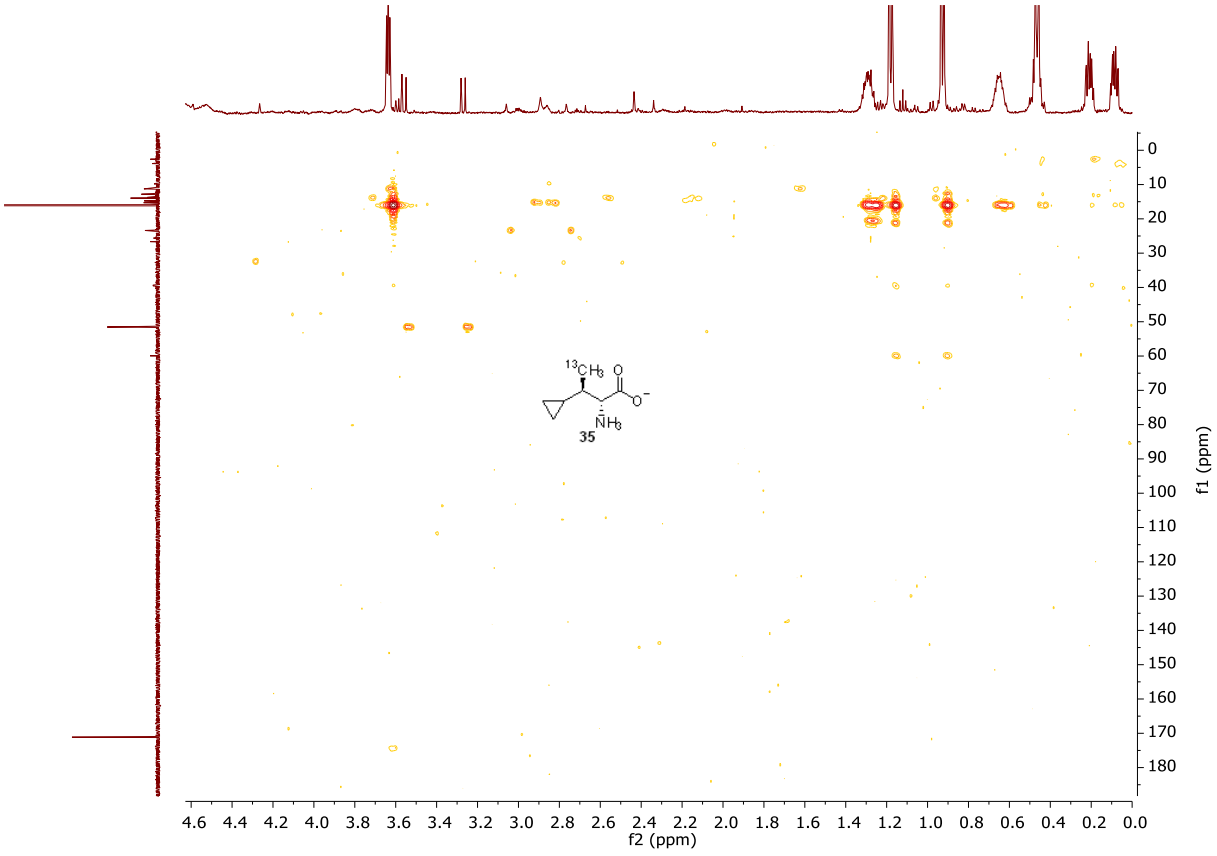
1.33
1.32
1.32
1.31
1.29
1.09
1.08
0.71
0.70
0.69
0.68
0.67
0.66
0.66
0.64
0.53
0.52
0.51
0.49
0.48
0.46
0.26
0.25
0.24
0.23
0.22
0.14
0.13
0.12
0.11
0.10
34
0.96
1.24
2.71
1.11
2.10
1.04
0.99
f1 (ppm)

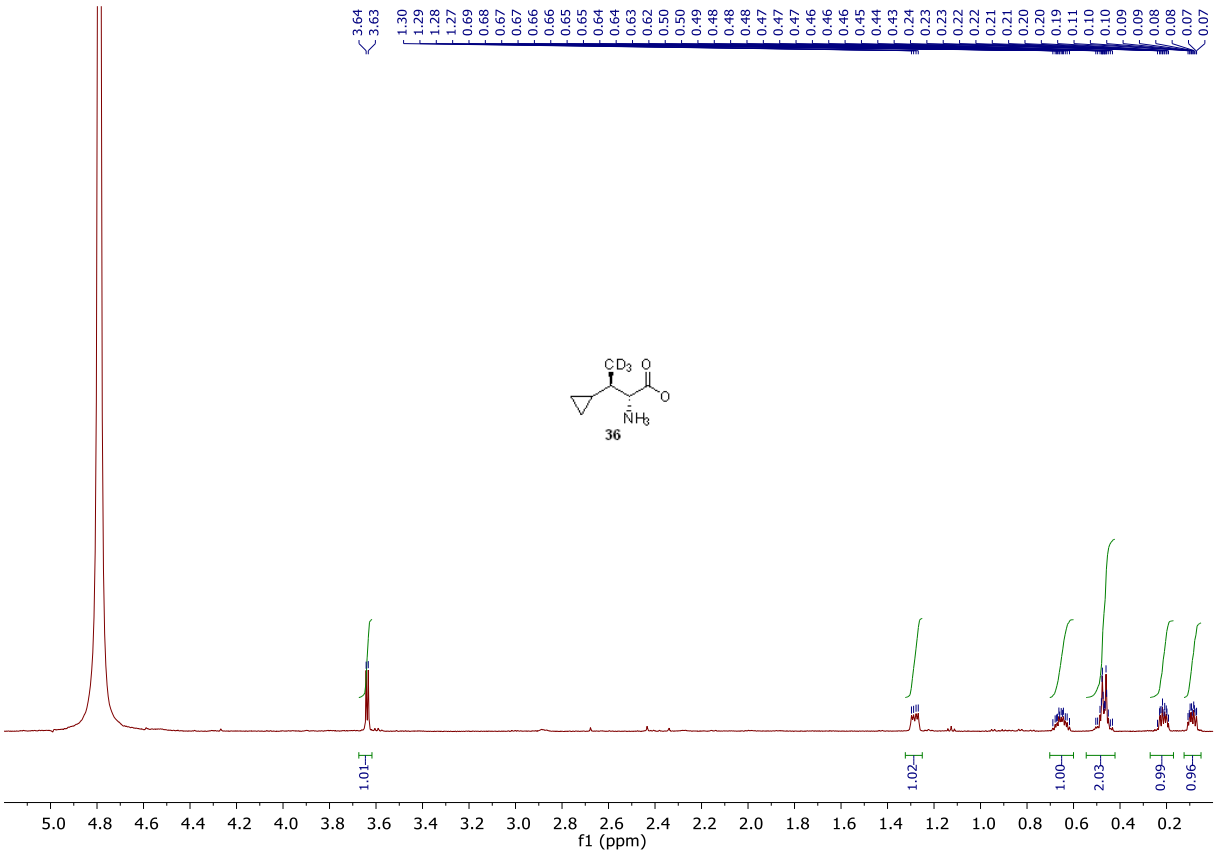

59.99
39.49
16.02
12.81
3.88
2.59
34
f1 (ppm)
f2 (ppm)

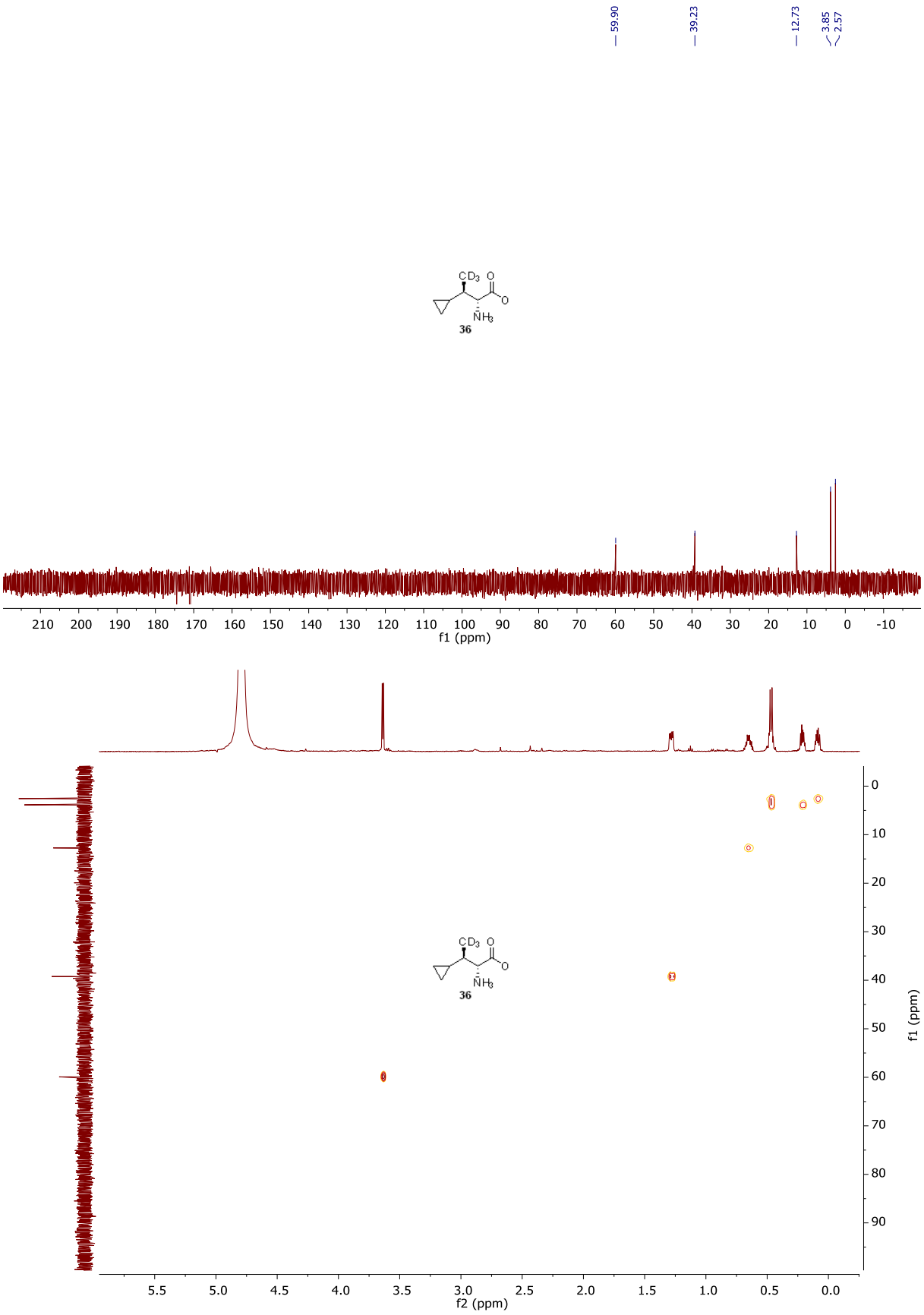

34
f2 (ppm)
f1 (ppm)

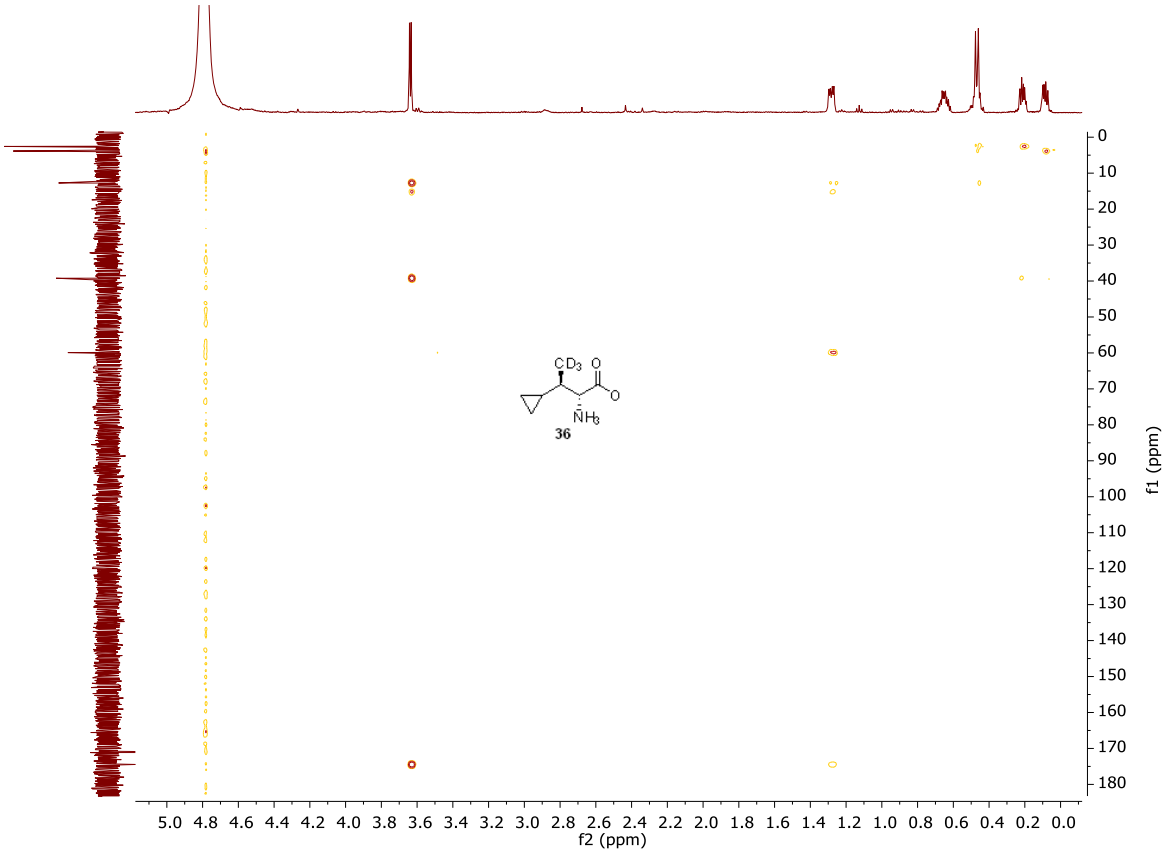

35
1.00
1.04
1.50
1.52
1.03
2.09
0.96
0.97
f1 (ppm)


59.93
39.58
39.30
16.01
12.81
3.89
2.60
35
f1 (ppm)
f2 (ppm)


35
f2 (ppm)
f1 (ppm)


36
1.01
1.02
1.00
2.03
0.99
0.96
f1 (ppm)


59.90
39.23
12.73
3.85
2.57
CD3
O
O
NH3
36
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
0 10 20 30 40 50 60 70 80 90
f1 (ppm)
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)


CD3
O
O
NH3
36
f1 (ppm)
f2 (ppm)

Supplementary References

1. Liao C, Seebeck FP. S-adenosylhomocysteine as a methyl transfer catalyst in biocatalytic methylation reactions. *Nature Catalysis* 2019, **2**(8)**:** 696-701.

2. Zou Y, Fang Q, Yin H, Liang Z, Kong D, Bai L*, et al*. Stereospecific biosynthesis of beta-methyltryptophan from (L)-tryptophan features a stereochemical switch. *Angewandte Chemie* 2013, **52**(49)**:** 12951-12955.